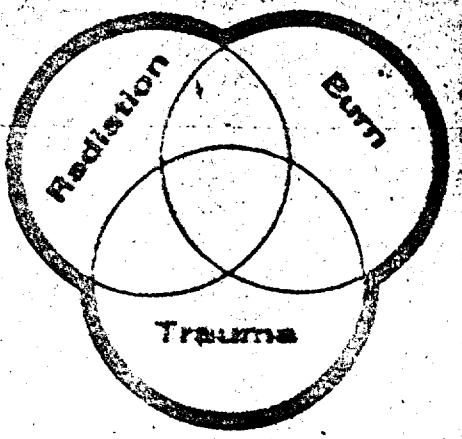
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The Pathophysiology of Combined Injury and Trauma



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AD-P003 880	Infection Prophylaxis in Large Patient Populations with Combined Injury.
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PATHOPHYSIOLOGY OF COMBINED INJURY AND TRAUMA

Proceedings of the First International Symposium held April 27-29, 1983 at the Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA.

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This report is the proceedings of the First International Symposium held 27-29 April 1983 in Bethesda, Maryland, sponsored by the Armed Forces Radiobiology Research Institute. The text is comprised of 29 research papers, which fall into three subject areas: Combined Injury and Trauma, Immunology and Inflammatory Responses, and Sepsis. Also included are an Executive Summary, a Panel Discussion summarizing the presentations, Abstracts of Poster Presentations, and a List of Attendees.

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EXECUTIVE SUMMARY

EXECUTIVE SUMMARY

The general medical term "trauma" is a simple six-letter word that is capable of conjuring up almost as many dreadful scenarios as there are individuals. The direct and ancillary effects of traumatic circumstances are staggering to comprehend. Trauma includes both accidental and intentional injuries, and it is the principal cause of death in Americans between the ages of 1 and 39 years. In 1982 alone, 165,000 trauma deaths occurred. The economic impact of trauma was more than $88 billion in 1980. Simple word? Yes. Tragic? Yes. Wide reaching and expensive? Yes.

Deaths due to trauma have been noted as presenting a trimodal distribution pattern. The first peak has been characterized as "immediate death," representing persons who die very soon after injury. These deaths are typically caused by lacerations of the brain or brain stem, upper spinal cord, heart, or one or more of the major blood vessels. The second peak, "early deaths," represents that segment of the population who die within a few hours of the injury, typically due to major internal hemorrhages and/or severe blood loss. The third peak is characterized by patients that die days or weeks after the original injury. In 80% of these late deaths, the cause is infection and/or multiple organ failure. This area may be one of the few in which interdiction is possible, and it is, in all likelihood, where our efforts should be applied.

The First International Symposium on the Pathophysiology of Combined Injury and Trauma has brought together the diverse backgrounds of over 100 physicians and scientists to focus on the complex problems engendered by injuries of a combined nature. It may be possible to develop a holistic approach to the management and therapy of combined injuries by marshalling the many talents of these investigators.

In the Symposium, speakers and discussants attempted to define derangements in homeostasis following polytrauma, including radiation injury. Different types of trauma were examined for pathogenic

commonality. Potential methodologies that were identified and examined included technical surgical problems and immunologic and biochemical alterations. These factors apparently predispose the patient to devastating infectious complications during the posttrauma period.

Trauma is a recognized fact of life in our highly industrialized and technical society. A pervasive problem in our nuclear era is the possibilty of nuclear disaster. Such an event could produce injuries of a nature and scale almost too horrible to contemplate. However, they must be contemplated, and we as physicians and scientists must be ready, even while making every effort to reduce the likelihood of that event.

SURGERY IN COMBINED INJURY

The chance of survival for a patient increases with the speed at which he is moved to a definitive care facility for further treatment. In spite of rapid treatment, sepsis and multiple organ failure often occur as late consequences of severe injury. The importance of debridement and wound closure in traumatic injury has been demonstrated experimentally in mice by Messerschmidt, who found that closure of a sublethal wound in mice given minimally lethal radiation doses reduced mortality from 90% to 18%.

Whole-body irradiation ($LD_{50/30}$) is not a contraindication for intestinal or retroperitoneal surgery if the surgery is carried out at the end of the period of acute radiation sickness. Early excision grafts are recommended in irradiated and burned subjects. It appears that surgical corrections should be performed within the first 48 hours, and elective procedures should be postponed until later in the convalescent period (i.e., 2-3 months).

Good trauma management necessitates the rapid removal of necrotic tissue and then wound closure. Wound closure is one of the most challenging of surgical areas in the combined-injury patient. Increased mortality occurs in the absence of wound closure. The problem appears to be that

any anticipated surgery must be performed within a very narrow time frame after injury. Conversely, we have learned only too well that closing contaminated wounds will have lethal consequences.

Many questions remain for future research consideration. Among them are: Should an effort be made at splenic repair rather than extirpation? What is the effectiveness of healing in a site of anastomosis after irradiation? Will different types of dressings or wound covers be more advantageous? Are there other methodologies that may be used in debridement procedures? Burn management in combined-injury patients is in itself a formidable task. Mass-casualty situations will present unique problems in which accuracy of triage is essential. Patients with third-degree burns in excess of 40% of the body surface area or total burns in excess of 60% of the body surface area require massive support. Ringer's lactate is recommended as the resuscitation fluid in patients having less than a 60% body-surface burn. Combined-injury situations in which radiation is involved present a very different picture. Survival is difficult in patients with 30% body burns coupled with radiation. That is why triage and personal dosimetry are vitally important with radiation casualties. Any contemplated dosimetry system for personnel must be simple, self-reading, and issued to everyone. A patient whose dosimetry shows survival potential should begin receiving treatment that has been adapted for initiation by untrained personnel, if necessary.

The British Falkland Island crisis demonstrated very low mortality rates and only limited infections in 730 patients. This maybe due to the use of early surgery and antimicrobial agents. Patients were given prophylactic doses of penicillin and tetanus toxoid, and wounds and burns were excised early and covered with dressings and silver sulfadiazine. Resuscitation fluid, when required, was Hartman's solution (1 liter) followed by the administration of colloid.

Renal failure often occurs with ischemia-induced trauma. This is due to an increased concentration of mitochondrial calcium. It may be prevented by calcium blockers such as verapamil.

Nutritional support is essential for effective recovery in the injured patient. Some attention was given to these nutritional effects by participants of the meeting. However, it is obvious that future meetings should examine more fully the multifaceted aspects of nutritional support.

MEDIATOR SYSTEMS

Combined injuries are associated with changes in levels or activities of many substances, including acute-phase proteins, immunosuppressive factors, prostaglandins, and coagulation factors. The reticuloendothelial system (RES) may be a common pathway in different forms of circulatory shock and trauma, and it may explain similar host responses. Macrophages and endothelial cells are known to be major sources of mediator molecules. The pharmacologic manipulation of these cellular systems represents a possible therapeutic opportunity, which remains to be exploited.

INFECTION

Antimicrobial agents have had limited impact on Gram-negative infection. Because of their limited impact, new approaches are needed for the effective management of complications concomitant with states of infection. Passive immunotherapy is one promising approach to the control of infection. Antiserum against core glycolipid of the J5 mutant of E. coli, common to many Gram-negative organisms, gives some protection against subsequent challenge with other Gram-negative organisms. Specific monoclonal antibodies directed against exotoxin A of Pseudomonas aeruginosa is known to enhance survivability in a murine burn model.

Identification of the effector and mediator molecules of immunosupression and shock associated with infection are much needed. Treatment of patients could then include the administration of pharmacologic agents to regulate mediator sustances. Other promising agents include calcium channel-blocking agents, antibiotics, and nonsteroidal antiinflammatory drugs.

Survival from shock has been noted as paralleling progressively improving RES phagocytic indices. For that reason, modulation of the RES system may be a worthwhile pursuit. Various natural and synthetic immunomodulators, now available, may prove useful for stimulating the RES. Immunomodulatory agents may not demonstrate the resistance problem particularly associated with antibiotics, since they are broad-spectrum.

Prevention of the abnormal colonization of mucosal surfaces of immunosuppressed hosts by opportunistic pathogens may significantly reduce infectious complications posttrauma. One of the most promising means now available for controlling colonization is the use of selective decontamination procedures. Selective decontamination uses poorly absorbed antibiotics to preserve anaerobic flora but eliminate potential pathogens. When systemic infection does occur, it is usually due to a mixture of organisms. Therefore it is advantageous to develop synergistic antimicrobial combinations to be directed against all components of mixed infections.

The low incidence of infections in patients injured in the Falklands crisis may be due to the fact that they were maintained in newly commissioned hospital ships. Nosocomial agents were either absent or were in extremely low concentrations. Our meetings in the future should consider how these conditions may affect the ultimate outcome of the patient.

USE OF BLOOD PRODUCTS

Fibronectin is purported to increase RES function and protect vascular beds. Decreased levels of fibronectin have been noted in instances of trauma. However, the significance of fibronectin to the well-being of the septic/trauma patient remains to be investigated.

Serum from burn patients is known to contain factors that are toxic to cellular functions. The technique of plasmapheresis has been used for this reason, and it has resulted in marked improvements in a number of patients. It too appears to be a technique deserving of more study.

Blood products and resuscitative solutions are vitally important in the treatment of shock patients. Red blood cells in combination with crystalloid/colloid solutions have produced satisfactory responses in some shock patients. Platelet concentrates and fresh frozen plasma have also shown results worthy of further investigation. Infusion of hematopoietic stem cell fractions may also prove to be of value. The goal is to have supplies of these frozen blood components readily available at central blood banks to supplement supplies of fresh blood, for use in emergency situations.

MODEL SYSTEMS FOR STUDY

It is imperative for correct models to be selected for the questions being asked. No system can be considered to be exactly similar to human systems, and because human studies are so limited, the appropriate animal models must be selected.

Better animal models for infection need to be developed. Rather than challenging healthy animals with massive doses of bacteria, compromised models are needed in which infection can be initiated with a small amount of inoculum, resulting in progressive infection. One such model, which is very promising, uses a plasma clot that contains bacteria; it is inserted into canines to mimic a progressively lethal infection. Similarly, rodent burn and radiation models have been established that may offer realistic models for the study of infections in an immunocompromised host.

COMBINED INJURY AND TRAUMA

KEYNOTE ADDRESS: FIRST INTERNATIONAL SYMPOSIUM ON COMBINED INJURY AND TRAUMA

BACKGROUND: THE PRESSING NEED FOR NEW KNOWLEDGE ON RADIATION INJURY WITH PHYSICAL TRAUMA

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INTRODUCTION

The care of military and civilian casualties in the wars of the past century has defined new problems in pathophysiology, surgery, and medicine. These new problems have demanded new solutions. And in almost every case, these new solutions have reacted to the benefit of humanity throughout the world. New understandings of changes in the human body produced by various forms of injury have resulted in improved treatment of a whole variety of human illnesses. In addition, and rather unexpectedly, many of these advances have involved the development of wholly new concepts and methods in the biosciences.

This type of advance, occurring in conjunction with military casualties, has often given the erroneous impression to amateur historians that "surgical advance thrives on war." Nothing could be further from the case. It is merely that the accumulation of pressing problems in one place and at one time results in the pressures that drives human ingenuity: "Necessity is the mother of invention." Civilian disasters, be they earthquakes, fires, or explosions, have often resulted in the same sort of remarkable breakthroughs. It would be a shame if anyone hearing of this Conference were to get the wrongful idea that biomedical advances arising from massive nuclear warfare would in any sense counterbalance the human and global disasters resulting from such an occurrence.

The history of new advances arising from the recognition of new patterns of injury need not be belabored for a well-informed audience such as this.

It was Bywaters, during the London blitz, who perceived that after the weight was removed from seriously crushed limbs, individuals developed renal failure. "The crush syndrome" became the model upon which all of our early knowledge of post-traumatic renal failure (later called lower nephron nephrosis) was built. It is an amusing irony of history that it was the lifting of weight from crushed limbs that led Walter Cannon (in World War I) to the conclusion that fluid loss in injured tissue was at the basis of the then newly recognized syndrome of "shock."

Willem Kolff, working under the very eyes of the Nazis in the Netherlands, at about the same time that Bywaters was making his observations on renal failure, was developing a dialysis machine based on winding sausage casing on old tomato cans, a machine destined to save the life of thousands of people with post-traumatic renal insufficiency over the next few decades. This artificial kidney, as we understand so clearly now, was to become the leading alternative to kidney transplantation for patients with end-stage renal failure throughout the world.

Transplantation itself received a remarkable boost during World War II. Under the pressure of severely burned air force pilots, the British government undertook new research on methods of skin graft covering in burns. Gibson, an accomplished plastic surgeon in Glasgow, sought the help of a brilliant young biologist named Peter Medawar. The two of them published their first paper on skin homograft survival and clearly described the "second set" response. If a skin graft from one donor is placed on a recipient, it is rejected after a few days. If a second set of grafts is then taken from the same donor and put on the same recipient, it is rejected much more rapidly. Often the brilliance of a biologist is his ability to recognize in an unexpected finding the quintessential model of an important response.

Peter Medawar, later to be knighted for this work and to receive the Nobel Prize, recognized in the "second set" response an immunologic model for the study of immunogenetics and histocompatibility. From that knowledge came kidney transplantation as we know it today, but most importantly, came a tremendous explosion in the biochemistry of molecular genetics and molecular immunology.

Although shock was extensively studied in World War I and between the wars, it was its much clearer definition at the time of World War II that led Professor E. J. Cohn to start fractionating blood. His discovery that alcohol precipitation would allow him to fractionate human plasma without denaturing the protein, was a major advance of this century. Although concentrated human albumin saved many lives in World War II, it has almost disappeared from the scene save for occasional usage in liver failure. And yet, when anyone asks me about it, I cannot help but tell them the wonderful story of the day that I was having lunch with Professor Cohn. I was only a lowly research fellow at the time, and the data came in showing that each gram of concentrated albumin pulled into the circulation was exactly the amount of fluid that he had predicted from the differential osmotic pressure. At that moment Professor Cohn realized that concentrated albumin, hanging in a little vial from the belt of a corpsman, could do the work of five times its volume of plasma. From this work also came fibrin foam, fibrinogen, the clotting factors, and then later the separation of platelets, white cells, immune globulins, and a whole bevy of important functions from either whole blood or plasma. Another amusing story of the era was a meeting of the National Research Council held in Washington in about 1945, where a distinguished professor reported that after many months of work, he had found that red cells were best suspended, and survived best, when all of the known fractions of plasma were added to the mixture! I am sure that someone with an evolutionary turn of mind might have said, "Well, blood evolved that way; why did you have to waste so much time rediscovering it?"

The first use of chlorine gas as a mass toxin was carried out at Ypres in Belgium in 1915. It produced an exudative bronchopulmonary secretory response that resulted in flooding of the lung with body fluids and death in severe cases. At the time, not much could be made of this, and no treatment could be evolved. The gas mask was made available and became standard equipment. It is another historical irony of this century that the lesson of how to handle low pressure pulmonary edema was not really learned for another 40 years, and that even today, we are not sure how best to adjust respiratory assistance apparatus for this type of exudative response. But the response was recognized. And at several civilian disasters (the Coconut Grove Fire in Boston in 1942 being a spectacular example), pathologists recognized changes in the lungs of those who died in the fire (and were totally unburned) as showing the exudative changes

of poisoning during World War I. It is also a remarkable fact of this century that poison gas was not used at all in World War II; its use was either zero or so localized as to have attracted no historical attention. In our anxiety to avoid an exchange of nuclear weapons in some future conflict, we should try to understand the military decision-making process and the political pressures that avoided the use of gas in World War II, when it had been one of the most terrifying weapons of World War I.

The development of the submarine might never have been considered to have anything to do with pulmonary function. But it was the deep water submarine tank at the Naval Research Institute that led Otto Behnke to his gravimetric methods of analyzing body composition, and then later long series of studies (many of them carried out at the submarine bases at New London and Groton, Connecticut) on pulmonary physiology. The particular challenges of sudden changes in pressure in the airway were felt by the submariners just as they were also felt on explosive decompression during air warfare.

The development of vascular surgery must be added to this list. The extensive vascular surgery of World War II, so carefully analyzed by Dr. DeBakey and his colleagues, would now be regarded as primitive. Direct replacement of arteries by homografts, allografts, and prostheses came along within months of the end of World War II. By 1950, Professor Charles Rob, at Saint Mary's Hospital in London, had dozens of patients with aortic replacement. Direct replacement of arteries and veins formed major surgical advances that were useful to patients throughout the world, and of course they were of major military application in the conflicts of Korea and Vietnam.

MIXED RADIOBIOLOGIC AND PHYSICAL INJURY

The foregoing account of past achievements could be expanded historically, but we are on the verge of a new challenge, and it is certainly more appropriate to look to the future than the past, especially for a seminar such as this.

Mixed radiobiologic and physical injury has been seen on a grand scale only twice in the history of our planet: in Hiroshima and Nagasaki. Most of the data developed from those episodes were brought together by the Atomic Bomb Casualty Commission of the U.S.A. in collaboration with the Japanese, and later were taken over with increasing authority by the Japanese themselves. Most of the information deals with late effects. Data on early management are very scarce largely because early medical management was almost non-existent. The hospitals and other medical facilities of both cities were destroyed, and many of the physicians were killed by the initial explosions.

Much has been made of these devastating effects in a medical context. One school holds them up as an example to the world, demonstrating that atomic warfare should never occur again, a point of view with which few could disagree, whatever its predictive significance.

Others have drawn a different conclusion, namely that if an individual could protect himself or herself from blast heat and radiation by a shallow earth shelter and minor coverage, injury would be avoided. This view, together with massive relocation of population, is, in my opinion, equally unrealistic. The exact timing of an attack or an explosion is never known for sure. Exactly when are you to dig your grave and lie in it? The massive relocation of people neglects completely the problems of food, water, sewage, civilian crime, and municipal organization. It also neglects the fact that in the outer perimeter of the blasts at Hiroshima and Nagasaki, the people who were least injured were those who were deep in cellars or in their own homes, protected at the margin of blast and radiation injury by the very structures that they would be forced to leave if relocation had been the policy.

Despite the limitation of our knowledge from those two episodes, their further study and computer projections of blast and radiation effects are of basic importance to the mission of this meeting, although in a logistical sense rather than physiological.

On a microscale, combined radiobiological and physical injury has been seen in two categorical circumstances since 1945. The first of these is the occasional radiation accident. Most of these have been incidental to the

management of atomic fuels and fission energy, usually in an industrial or manufacturing center, and usually of the atomic pile variety rather than a cyclotron or an exposion. These industrial accidents have been important experiences, and will doubtless be reviewed at this meeting. The lessons of Three Mile Island and the nuclear industry are yet to be learned.

The second category have been those in which whole-body irradiation has been given intentionally, either as a feature of the treatment of lymphoma, leukemia, and Hodgkin's disease, or as a preparation for the transplantation of kidneys, liver or bone marrow. In the former instance (that is, the use of whole-body irradiation or at the very least, widespread bodily radiation in the treatment of lymphoma), many effects have been seen that have been helpful and valuable in understanding radiobiologic injury and its combination with physical trauma. However, in the kidney transplant and bone marrow transplant setting, a surgical procedure that is very major in the former and rather minor in the latter, has sometimes been superimposed upon radiation at a varying time interval. Speaking from my own experience, it was this combination of whole-body irradiation with the surgery required for kidney transplantation, that first alerted me to the biological challenge imposed by the combination. Our patients were few in number. Whole-body irradiation was given in a very simple geometrical arrangement. The radiation dose was loosely calculated without the accurate measurements available today. The dosages were between 300 and 800 rads given in either one, two, or three sittings.

The operative procedure for transplantation occurred within a matter of days after the radiotherapy. The original concept was that the valley of immunosuppression due to radiation might coincide roughly with the predicted first rejection episode of the kidney, and that the improvement in kidney function would help the patient "weather through" the second wave of radiation injury consisting of bone marrow suppression.

To the extent that kidney rejection was abated by radiotherapy, the effect was reasonably successful. As you probably know, other experiments were carried out and have been since, in which the local kidney or other specific anatomical regions (specifically those involved with lymph nodes) have been

radiated, with less drastic systemic effects. However, whole-body irradiation then proceeded to take its disastrous toll, and over the course of the next days, weeks, or months, the patient began to pay the price of this massive continuing injury.

In one patient, success was achieved. The whole-body irradiation dose was a little less than the others, estimated at 450 rads. The donor was closely related, a fraternal twin. Although this genetic similarity would favor acceptance of the transplant (although it would fail to guarantee it as in identical twins), it would have little effect on the severity of the irradiation-induced illness. By whatever combination of surgical care and good luck, success was achieved despite the awesome combination of whole-body irradiation and a major operation.

Death was due in all cases to a terminal infection. We have long since learned to recognize in critical care medicine and in severely injured patients that the terminal infection that finally carries the patient off should not in any sense be regarded as the proximate cause of death. The problem always is one of antecedents: Why is the infection there, and what makes the patient prone to the infection? The mere diagnosis of "multiple organ failure" tells us absolutely nothing. The question is, "What has gone wrong with the immune defenses or the biochemical feedback regulators that normally govern the body?"

The decline in formed elements of the blood was obvious. Less apparent were the subtle changes in immune function, many of which could not be measured or quantified in 1956 with the elegance that they are now, almost 30 years later. Without trying to reconstruct these cases in detail, the fact remains that a breakdown in immunity and an apparent defect in healing as well as a tendency to very early deterioration in lung and liver function, could certainly not be abated by the rapidly improving kidney function, even though the patient had chronic renal failure. Whole-body irradiation has been used since that time for a number of different purposes. The very large fields used in the treatment of Hodgkin's disease cannot be equated with massive whole-body irradiation, even though there are doubtless many changes in common. The use of whole-body irradiation for bone marrow transplantation with or without immunosuppressive drugs always supplies a model.

CIVILIAN AND MILITARY PROJECTIONS AND SETTINGS

This Conference has been called to review not only the known effects, but the development of suitable laboratory models for the study of combined injury of this type. The pathophysiologic scope of the problem is evident from the program of this Conference, and to review it further here would be redundant.

From what little we know, civilian settings of combined radiobiological and physical injury are chiefly confined to workers in the nuclear energy field, or, in the case of a nuclear energy industrial plant disaster, some of the local residents of the area. A second possible vulnerable group would be those engaged in the production, management, deployment, transportation, maintenance, or supervision of nuclear missiles, particularly those involving the element plutonium.

From the military side, others here today can predict the setting far better than I. It seems superficially evident that we should consider as distinct the settings and epidemiology of combined injury on the battlefield; tactical situations where geographical scope might be limited, and the nature of the radiation might in some cases be almost monovalent, consisting, for example, largely of neutrons. Whatever the details there will be a central core of hopeless injury, an outer periphery of readily salvaged persons, and a critical torus, or doughnut shaped zone, where injury is very severe, often combined, and survival unlikely, but conceivable. It is to that group of patients that this Conference is particularly directed.

In the event of the ultimate intercontinental ballistic missile catastrophe that we all apprehend, should there be a major would conflict involving nuclear exchanges, there will be far more destruction. But we will be faced with the same triphasic zone arrangement of an inner lethal area, an outer fringe of salvageability, and a torus of medical challenge. We will have the additional problem, possibly not so evident in the battlefield situation, that the medical facilities of the area will be largely destroyed. Therefore, somewhere down the line, we are going to be thinking about first aid or measures that might be taken by unskilled personnel, self-help and public education.

It is a tribute to the Department of Defense and to the preparedness plans of our nation that this Conference has been called. There are many pessimists, activists, and propogandists who feel that a Conference of this type, somehow by its very nature, admits the possibility of and therefore encourages atomic warfare. I could not disagree more wholeheartedly with such a Madison Avenue or media view. It is our job to look after the sick and injured, either of the military or civilian populations, and of all ages and all occupations or degrees of vulnerability, whatever the public relations impact may be. We must do our job even if people say it is misleading to indicate its necessity. If some say that a conference such as this invites nuclear war, then we are entitled to ask if the writings of Ambroise Pare brought on the invention of dynamite. In the words of the Prophet, let us "Hew to the line, let the chips fall where they may."

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PATHOPHYSIOLOGY OF COMBINED INJURY AND TRAUMA: AN OVERVIEW

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INTRODUCTION

Combined injury (multiple trauma, thermal burn, radiation) is a major medical problem in both clinical practice and military medicine. Trauma is the chief cause of death in people less than 38 years old, accounting for more than 150,000 deaths in 1980. The overall death rate for young American adults is far greater than most other modern western countries. The social cost of the death of these young productive persons is enormous. What is most tragic is that optimal trauma treatment could prevent at least half of those deaths. The American College of Surgeons Committee on Trauma has presented guidelines for optimal care, which can be summarized as getting the right patient to the right hospital at the right time.[1] Many of the lessons learned on the battlefield were used in developing these guidelines.

The increased utilization of radioisotopes and radiation devices in industry, science, and medicine necessitates their transport via highways, rail systems, or air transport. The possibility exists that any of these systems may become involved in an accident and result in casualties with a combined injury (radiation and trauma). Radiation-induced injuries can be a more serious clinical problem when a larger number of casualties is involved or when a combined injury is present. Instability in world politics and the marked proliferation of nuclear weapons have increased the probability that health professionals will have to treat combined injuries.

A recent report notes that the Nuclear Emergency Search Team has responded to many threats from terrorists and extortionists since 1976.[2] By the end of this decade, approximately 30 countries will have nuclear weapons. The medical community must be prepared to treat radiation casualties

regardless of whether caused by a radiation device, medical misadventure, reactor accident, terrorist team, or third-world power exercising its nuclear capability.

It has been 15 years since the problem of combined injuries has been reviewed.[3] Since then a great deal has been learned about the pathophysiology of combined injuries. This proceedings will review the mechanisms in combined injuries, sepsis, immunological and inflammatory responses, surgery and wound healing, and therapy. This paper will briefly review experimental data on combined injury, and try to provide an overview of the primary pathologic processes causing death in trauma, radiation, and combined injuries. Other papers in this proceedings will definitively describe specific aspects in pathogenesis.

RADIATION INJURY

The acute radiation syndrome is normally divided into four phases: prodrome, latent, manifest illness, and recovery. The prodrome, which begins within hours of exposure, is an initial toxic period. The effects are probably due to acute radionecrosis, with the release of vasoactive substances and other neurohumoral agents causing systemic manifestations. Signs and symptoms include anorexia, nausea, vomiting, diarrhea, intestinal cramps, salivation, and dehydration produced by the gastrointestinal system. Fatigue, apathy, sweating, fever, headache, and hypotension followed by cardiovascular shock may be produced by the neurovascular system. A subsequent pancytopenia may result, which causes a profound immunoparalysis and susceptibility to infection and hemorrhage.

Radiation injuries in the future may include the hazards associated with transport and use of radioactive material in peacetime or the comuination of nuclear weapons with chemical, biological, or conventional warfare. The biological effects induced by these combined injuries will markedly increase the casualty burden and will significantly impact the patient's ability to survive and recover. Patients with conventional trauma and irradiation at Hiroshima and Nagasaki developed significant complications at 2 to 3 weeks

after exposure, corresponding to the time of hematopoietic depression. Open wounds of many patients stopped healing, lacked granulation tissue, and hemorrhaged. These patients lost weight, and many died of sepsis. While support systems for the care of the critically ill patient or trauma patient have increased the chances for survival, it will be difficult to predict at the time of triage which patients with traumatic injuries will develop radiation sickness. The prodromal symptoms, which are useful in assessing the radiation exposure, may be unreliable when occurring in association with conventional trauma. Other than the victims in Hiroshima and Nagasaki, there has been little experience in the management of radiation injuries and associated trauma.

The hemopoietic system is very sensitive to radiation. Sublethal doses can cause significant and immediate reductions in hemopoietic stem cell and progenitor cell populations. The consequent decrease in mature functional cells such as neutrophils, monocytes, and lymphocyte populations occurs within the first week after exposure. It is during this period that the patient is at great risk. Those functional cells are necessary to initiate an integrated cell-mediated response to opportunistic pathogens and/or to complete the wound-healing process, but they may be reduced to ineffectual levels by irradiation. This combination of radiation plus trauma produces a unique set of problems for the traumatologist. In most instances, either the necessity for emergency surgical procedures or the signs and symptoms associated with the particular traumatic event, or both, will mask the first reliable indications of radiation injury. Life-threatening exposure to acute whole-body irradiation has been so infrequent that strategies for treatment must be devised from the few actual cases, the experimental animal studies, and the analogous states of clinical diseases. Regardless of the type of exposure or contamination, the physician's initial response to a patient with radiation injury is the life-saving treatment of nonradiation injuries. The first actions must be the standard emergency medical procedures: Insure that the patient has an open airway and is ventilated, that adequate perfusion is maintained, and that life-threatening hemorrhage has been stopped. It is essential to remember that radiation injuries are not acutely life threatening and that traumatic and burn injuries are likely to be more important. Once

the patient is stabilized, decontamination may be initiated. When feasible, decontamination may be performed simultaneously with treatment. Assessment of the radiation injury, including time of exposure, total dose, and whole-body or partial-body exposure, can then proceed. All available information must be gathered in order to aid the physician in determining secondary care due to radiation-induced aplasia and potential immune suppression.

COMBINED INJURY AND TRAUMA

Trauma elicits a predictable physiologic response. However, there are important specific effects of trauma that significantly alter the patient's response to both the trauma and the subsequent treatment, which alters the risk of complications and the injury-associated mortality. Battlefield casualties may result from multiple wounding agents along with the occurrence of simultaneous burn, blast, radiation, and mechanical trauma. Trauma-specific effects include the destruction of tissue and the associated liberation of vasoactive substances from burns, velocity-related tissue effects of penetrating missiles, pulmonary and gastrointestinal effects of blast, occult tissue damage secondary to blunt trauma, and gastrointestinal and hematopoietic effects of radiation injury. Each traumatic injury requires specific physiologically based resuscitation and wound care in order to minimize morbidity and mortality.

Injuries that are benign by themselves will become lethal when combined with relatively small doses of whole-body irradiation. This combination produces a unique set of problems for the physician. In most instances, the need for emergency surgical procedures or the signs and symptoms associated with the particular traumatic event will mask the first reliable indications of radiation injury. Sheep exposed to a mixed neutron and gamma dose of 400 rads and 1 hour later subjected to an abrupt overpressure suffered increased mortality from 25% to 50%.[4] Messerschmidt has summarized much combined-injury data; he stated that an open wound markedly increases the chances of infection, and recommends immediate closure of the wound.[5] Open skin wounds created on the backs of mice 1 to 4 days before irradiation, which increased the healing time from 16 to 18 days. When the wounding occurred within 2

hours of irradiation, healing time increased from 16 to 24 days. This and other experiments have shown the significant effect of the presence and timing of irradiation on all components of wound healing. Mortality from radiation in mice rose from 26% to 90% when combined with open wounds on their backs. If the wounds were immediately closed, mortality was 18%.[5] This last observation of mortality (decreasing or remaining unchanged when the wound is immediately closed) is very important, for it suggests that some mediator or process is turned off.

Burns associated with radiation cause synergistic increases in mortality. A contact burn was applied to mice before and after 510 rads whole-body irradiation. The radiation exposure had a 10% mortality and the contact burn had a 30% mortality.[6] The combination of these injuries resulted in an increase in mortality to 90%. Brooks et al.[7] showed that as little as 25 rads increased the mortality in dogs with a burn of 20% body surface area; the combination of 100 rads irradiation with a burn of 20% body surface area increased mortality from 12% to 75%.

The impact of whole-body irradiation on the healing of a bone fracture is also impaired. Zemljanoj[8] followed callus formation radiographically in rabbits from which a piece of subperiostial radius had been removed 2 hours after receiving 800 rads (LD20/30) irradiation. The control-group rabbits were completely healed by day 32 whereas the irradiated animals did not show complete healing until 60 days postirradiation.

Early closure of wounds is desired in combined injuries of soft tissue, but it is not always possible or even then proscribed in most surgical situations. Normally an open wound is exposed to colonization and possible systemic sepsis, conventional soft-tissue wounds in the patient with combined injuries may significantly enhance the development of sepsis. The immunoparalysis caused by combined injuries is much more severe than the trauma from a single injury. The bone marrow stem cells are very sensitive to radiation and may require more than a month to recover; during this time, the patient's immune system is dangerously depressed. Ionizing radiation compromises the patient with respect to his natural defense against exogenous infectious

agents, endogenous gut-derived bacteria, and their associated toxins. An early phase of nonspecific cell-mediated resistance plays a vital role in the first line of defense against bacterial disease.

The major part of early resistance to infection is determined by the ability of both the circulating and the tissue granulocytes and macrophages to kill invading microorganisms and to release a variety of immunostimulatory and hematopoietic factors. Macrophages are relatively radioresistant and survive for long periods of time in situ. It follows that their function during periods of leukopenia after radiation injury is important.

Injuries to the abdomen also present many potentially significant problems in the irradiated patient. Blast overpressure, blunt trauma, and penetrating wounds are all significant causes of abdominal injury. Messerschmidt[9] evaluated the impact of laparotomy or splenectomy in mice receiving whole-body irradiation. Radiation with 510 rads alone caused mortality of 27% whereas the laparotomy or splenectomy alone caused mortality of about 5%. Splenectomy at 2, 4, or 8 days postirradiation increased the mortality to 60%, 75% and 85%, respectively. Laparotomy alone combined with irradiation caused a maximum mortality of 50% when the surgery was performed on day 8. The role of the spleen in enhancing the ability to resist bacterial infections has been amply demonstrated in recent years.[10]

SEPSIS

Despite the proliferation of antibiotics and the employment of early definitive surgery, sepsis remains the most frequent cause of complications and death in patients who survive their injury and initial surgery. A variety of microbial factors is important in the pathogenesis of sepsis, such as adherence properties, motility, and production of enzymes and toxins. These factors have been found to be important in terms of tissue destruction and systemic invasion. It is important to remember that the presence of opportunistic pathogens that have selective mechanisms of invasion in the irradiated host significantly increases the risk of sepsis due to ineffective

cell-mediated host defense mechanisms. The mature cells responsible for chemotaxis, phagocytosis, microcidal and mediator functions may be compromised by radiation-induced defects.

Sepsis posttrauma has primarily resulted from Gram-negative organisms, and in the last 10 years, anaerobic organisms have been noted as frequent pathogens. Shires and Dineen assert that the major determinants of sepsis and bacterial invasiveness are the degree of injury, magnitude of external contamination, organism invasiveness, host resistance, and endogenous organism invasiveness.[11]

Multiple systems failure is often the final result of severe trauma and its septic complications. The respiratory distress syndrome was the most frequent life-threatening complication in casualties during the Vietnam War, and is the most frequent cause of death in severely injured patients who develop postresuscitation infections. This complication of trauma and combined injuries may occur at any time after injury.

Numerous pathogenic factors have been implicated in the etiology of posttraumatic pulmonary failure. These include physical injury of the pulmonary parenchyma by blast or inhalation injury; effects of fluid resuscitation volume; composition of resuscitation fluids; and liberation of vasoactive mediators such as histamine, serotonin, and prostaglandins. Immunologic changes may also alter pulmonary function as a result of activation of complement, the formation of white cell aggregates, and the liberation of lysosomal enzymes that damage the capillary endothelium. The frequent association of respiratory failure with sepsis has focused attention on both the direct and indirect effects of microorganisms on the lung. Host and microbial factors that have been implicated in lung injury include microbial enzymes, microbial toxins (e.g., endotoxin), activation of complement, release of tissue-destructing enzymes from granulocytes and macrophages, and alpha-2 surface-binding globulin (fibronectin).

IMMUNOLOGICAL AND INFLAMMATORY RESPONSES

Immune defenses are decreased following burn, trauma, and radiation. The principal sequelae of traumatically induced immunosuppression is sepsis. The immunologic system is divided into the humoral and cell-mediated categories. Humoral antibodies in conjunction with complement neutralize viral and bacterial toxins, opsonize bacteria, and mediate chemotaxis of macrophages and granulocytes. Cell-mediated immunity is responsible for organ graft rejection, tumor immunity, and reactions to infectious agents. The humoral and cell-mediated immune systems cooperate in the immune response to foreign antigens. Granulocytes are also important to host defense against infection. These cells have defective intracellular killing activity after thermal trauma. When the burn wound was covered with autologous skin, the neutrophil function returned to normal.[12] Severe battle trauma has produced similar defects in neutrophil function during the first 24 hours after injury.[13]

The reticuloendothelial system also demonstrates diminished capability posttrauma. Saba has extensively studied the effects of trauma on the reticuloendothelial system (RES) and demonstrated markedly decreased phagocytosis as well as diminished levels of an alpha-2-globulin opsonin (fibronectin).[14] Infusion of purified opsonic protein prevents depressed RES function. In addition to opsonizing Gram-positive bacteria, fibronectin also helps phagocytes clear debris from devitalized tissue, mediates attachment of bacteria to injured tissue, and mediates platelet activation.[15]

The role of the macrophage in cellular immunity and hence its response to trauma have been better realized over the last decade. The macrophage is primarily a secretory cell. It has the capacity to synthesize and release a staggering array of factors that can mediate, induce, activate, and suppress specific aspects of the cell-mediated immune and hemopoietic responses to trauma and sepsis in the normal and irradiated host. It is also a remarkably efficient phagocytic cell, and has three well defined functions.[16] It is responsible for normal tissue debridement. The macrophage also phagocytizes microorganisms. Finally, it interacts with the lymphocyte to induce a response to antigens as well as induce expression of cell-mediated immunity.

Unlike granulocytes, the macrophage extrudes bacterial protein on its surface for immunologic processing of the antigen by T lymphocytes (cell-mediated immunity). When the immune system is activated, a chain of mediators signals the macrophage and T lymphocyte to activate B lymphocyte conversion into plasma cells, which produce immunoglobulin against the expressed antigen. This elegantly orchestrated system results in a rapid and efficient mechanism for fighting bacterial invasion.

Depressed humoral and cellular immunity is a serious problem after traumatic or combined injury. Munster postulated the existence within 24 hours after trauma of a suppressor T cell system activated against other immune elements, including normal T cells.[17] The net result of this is a diminished response to infectious challenge, resulting in diminshed cell-mediated immunity and antibody production. Ninneman et al. showed markedly diminished cellular immunity and prolonged human skin allograft survival in severely burned patients.[18]

Just as important as increased suppressor T cells is the decrease in immunoglobulins postirradiation and posttrauma. Immunoglobulins are glycoproteins that are produced by B lymphocytes in response to antigenic challenge. B cells produce five classes of immunoglobulins, the most important of which are the IgG, IgM, and IgA. IgG is the most important because it is the opsonizing antibody for most Gram-negative and Gram-positive organisms. Decreases in all serum antibodies have been reported in burn patients.[19] Antibodies not only opsonize antigens but also initiate the fixation and activation of complement.

The classical and alternate complement pathways involve discrete proteins present in all normal serum. These proteins interact in a precise sequence to elaborate biological activities that may augment host defense or injure host tissues. The classical complement pathway is activated by antigen-antibody complexes. Activation of complement releases mediators that participate in many activities such as augmentation of host defenses, chemotaxis, immune adherence, and phagocytosis. In the absence of antigen-antibody complexes, the activation may be via the alternate pathway. Release of complement

components causes a release of many vasoactive substances from mast cells (especially histamine), which increase vascular permeability and bring more antibody and complement to the sites of injury. This process can be deranged easily, with profound circulatory consequences. It should be noted that significant increases in histamine and prostaglandin occur postirradiation and almost certainly after combined injury.[20,21]

Great strides have been made in deciphering the various immunoregulatory, helper, effector, and antibody-mediated aspects of the immune system. However, few data exist on the effects of radiation on these components and the subsequent immune status of the irradiated animals. This is especially true for the larger mammalian species such as the canine and primate.

The source of all immunoregulatory cells is the bone marrow. The integrity of the marrow postirradiation, as a viable hemopoietic organ, determines the ultimate survival of the animal. If the total dose received is sublethal, two other considerations must be examined. First is the time required for regeneration of the hemopoietic stem and progenitor cells and the consequent period of time during which functional neutrophils, monocytes, and lymphocytes will be diminished. Second is the effect of combined trauma and/or sepsis on the recovery of the irradiation-damaged hemopoietic system and replenishment of functional white cells is very important. Little is known concerning the effects of various mediators on regeneration of the hemopoietic system in trauma-induced, immunosuppressed animals. There is no doubt that stimulatory factors are released and that mobilization of granulocyte-macrophage progenitor cells can be accomplished in animals and humans subjected to mild or nonimmunosuppressive trauma.[22-24] The more pertinent question concerns the hemopoietic effects following episodes of trauma that are immunosuppressive and life threatening. Survival following exposure to radiation doses and trauma that induce hemopoietic and immune dysfunction will depend on the effective use of therapeutic interventions. Success in defining effective therapeutic modalities will depend on an active research program concerning hemopoietic stem cell physiology, cell-mediated and humoral immune systems, and mechanisms of pathogensis of opportunistic pathogens.

SURGERY AND WOUND HEALING

Care of the wound to facilitate and enhance wound healing is the central theme of postresuscitation management following injury. The assessment of tissue viability to guide the surgical excision of necrotic tissue, yet conserve all viable tissue and limit tissue and functional loss, is imperfect in trauma alone. In combined injuries involving radiation, wound healing will be markedly delayed, significantly complicating the determination of tissue viability and debridement.

The control of blood loss is important in modifying both systemic and local reactions to injury, in wound healing, and in reducing the risk of infection in the postoperative period. The development of shock, secondary to a decrease of circulating blood volume, is the most frequent cause of early death in the severely injured battlefield casualty. Appropriate resuscitation during this critical period can influence the outcome, whereas inappropriate resuscitation will exaggerate the pathophysiologic changes that occur and produce later complications, increasing both morbidity and mortality.

Since the organ, tissue, and cellular effects of shock are directly related to its duration, means to prolong the interval between onset and irreversibility need to be developed to increase salvage in an environment of limited resources. Identification of the maximum depression of cardiovascular function associated with salvage is required for assisting in triage and for promoting effective medical and surgical care in the exigent situation. The need for the identification is exacerbated by the accelerated shock syndrome seen in combined injuries.[25]

THERAPY

The gastrointestinal tract responds to injury by the cessation of function in terms of both decreased motility and altered movement of fluid, nutrients, and electrolytes across the mucosal barrier. These pathophysiologic responses can be further aggravated by direct injury to the gastrointestinal tract and subsequent perforation. Radiation will significantly

exacerbate these problems because of the exquisite radiosensitivity of intestinal epithelial stem cells. Immediate postinjury ileus obviates oral resuscitation except in the case of the most trivial injuries. The loss of a major portion of the small intestines is a severely crippling injury or is potentially fatal, and means to protect the remaining surface area is a clear concern to traumatologists.

Stress ulceration is the most frequent life-threatening gastrointestinal complication in trauma. Although the use of antacid or H_2 receptor antagonist prophylaxis has reduced the incidence of this complication, such treatment is imperfect. Improved means to prevent or treat gastrointestinal mucosal injury and enhance mucosal repair is essential, especially with concomitant radiation injury. The mucosal response to injury is universal throughout the gastrointestinal tract, particularly those with infection. Stress ulcers have been found in the distal small bowels and the colons of severely injured patients. It is essential to develop effective prophylaxis and treatment to reduce the morbidity and mortality resulting from the gastrointestinal complications following combined injury.

Acute renal failure may occur as a result of prolonged hypovolemic or endotoxin shock and adversely affect patient outcome. Hemodialysis and peritoneal dialysis have improved the survival of patients with this complication, but little improvement in overall salvage has been realized since dialysis was first used in the Korean conflict. The early diagnosis of acute renal failure, with modification of fluid management and development of pharmacologic regimens, is necessary for prophylaxis and/or treatment that will modify the renal effects of hypovolemic shock or the secondary vascular alterations that occur in kidneys with established renal failure.

Evaluation of both the early and delayed effects of noncolloid and colloid-containing fluids for specific injuries is required in order to reduce complications of such treatment, particularly with regard to late occurring pulmonary, cerebral, renal, and septic complications. Since there are specific limits of asanguinous fluid replacement, there is a critical need to evaluate the clinical usefulness and safety of blood substitutes such as

stroma-free hemoglobin and fluorocarbon solutions. The use of oral fluid resuscitation is markedly limited in severely injured patients, yet resuscitation in an austere environment of limited medical resources may require such treatment. There is, therefore, a need to identify specific indications and limitations of oral resuscitation and, if possible, to develop solutions and techniques that will permit such resuscitation to be used for specific injuries in specific environments.

Various pharmacologic agents may ameliorate the effects of shock and hypotension. Further studies are required to assess the physiologic effects and clinical usefulness of such agents as prostaglandins or prostaglandin synthetase inhibitors, steroids, high-energy phosphate-containing compounds, and opiate antagonists. The use of prostaglandin synthetase inhibitors has already shown exciting promise in animal studies.[26]

Hypermetabolism accentuates the energy and nutrient needs of all injured patients. The magnitude of hypermetabolism and its duration are directly related to the severity of injury, and if the elevated nutritional needs of the injured patient are unmet, the patient's hospital course and ultimate outcome may be adversely affected. Research in the area of postinjury hypermetabolism has been limited by the difficulty in carrying out long-term studies in critically ill patients. Protein catabolism is particularly important to the immune system because catabolism of immunoglobulins may be accelerated as much as tenfold in severely burned patients.[27] Adequate nutrition must be maintained to provide adequate substrate for immunoglobulin synthesis.

Neurohormonal interactions are critically important in regulating postinjury metabolic changes. Research in this area is very active to define the initiators of postinjury hypermetabolism and identify the relative importance of central nervous system and hormonal changes in the response to injury, resuscitation, and sepsis. Nutrient utilization regulated by the neurohormonal changes must be optimized to disease-specific nutritional support regimens to reduce the total-body impact of postinjury catabolism, preserve lean body mass, and hasten convalescence. Organ-specific metabolic effects of combined

injury and infection must be studied in the liver, muscle, heart, and central nervous system in order to define physiologically sound regimens of organ function support.

Optimum injury-specific diets consisting of essential nutrients in efficiently utilized calorie/nitrogen ratios must be developed. Determination of the potential role and the clinical usefulness of hormonal treatment to modify posttraumatic hypermetabolism, catabolism, and accelerate the restoration of body composition during convalescence is necessary. Agents with promise include thyroid hormone, growth hormone, and steroids, as well as hormone antagonists such as adrenergic blockers used to counteract the catabolic effects of the catecholamines.

THE CHALLENGE

Two major points can be taken from the foregoing discussion of combined injury. First, much work remains to be done before we can fully understand and control the processes initiated by combined injury. The magnitude of this challenge should not make us pessimistic about the possibilities for achievement of this goal. In fact, many new discoveries made with regard to mechanisms and management of injury in recent years will be described at this meeting, as evidence of how far we have come.

Another fact we must consider is that future efforts in this exciting field will require a synthesis of the activities of all the multiple systems that interact in response to injury. For a truly comprehensive approach to be taken, the problems of surgery, cellular and systemic physiology, hematology, microbiology, coagulation, inflammation, and immunity must be pursued in models of combined injury. Development of strategies for regulation of host responses and enhancement of survival will have to consider all these components of combined injury and be themselves tested as combined therapies in realistic experimental models.

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RESULTS OF ANIMAL EXPERIMENTS AS A BASIS FOR RECOMMENDATIONS ON THERAPY OF COMBINED INJURIES (RADIATION INJURY PLUS WOUNDS)

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INTRODUCTION

With the dropping of the atomic bomb on Japan, combination injuries were caused for the first time in people due to the simultaneous action of whole-body irradiation with burns and wounds. However, the scientific information that reached us from Hiroshima and Nagasaki is highly insufficient, because in the unprecedented catastrophic situation in both cities, no error-free reporting could develop. In Hiroshima, 90% of the physicians were injured or killed; although in Nagasaki only a part of the city was destroyed, the university quarters with the clinics were hit, so that here also the greatest part of the medical profession was lost.

A further burden on many of the injured was the absence of all medical treatment. Many of the injured were lodged in the houses that remained standing; these were often burned out and without windows. On the ground, pressed together, lay women, children, old people, and soldiers, of which a great number were present at that time in Hiroshima. They had untreated, bleeding wounds, burns, infections, vomiting, and/or diarrhea. The additional stress of the complete absence of medical treatment must also be considered as a factor that caused combination injuries, as defined by us previously.

Only one precise statistical study is available about the occurrence of combination injuries in Hiroshima and Nagasaki. This study was provided by the "Joint Commission," an investigation group formed in October 1946, which consisted of American and Japanese physicians.[1] The Commission had selected 5,185 injured patients from H (Hiroshima) and 4,107 from N (Nagasaki), and determined the percentage of subjects who had only one, two, or three types of injuries. They observed 60.5% subjects in H and 57.7% in N with <u>one</u> kind of injury, 34.5% in H and 37.1% in N with <u>two</u> types of injuries, and 5.0% in H

and 5.2% in N with three types of injuries. The patients in the third group were injured by the effects of the blast wave, the heat radiation, and the nuclear radiation. The percentages in both cities are remarkably similar; however, they are valid only for patients who survived the effects of both nuclear exposions for at least 20 days. The patients who died within this time period were not considered in these statistics. A conservative estimate that includes all of those afflicted, both the survivors and the dead, seems to indicate that approximately half of all victims suffered from combined injuries.

Of the 5,185 "20-day survivors" from Hiroshima, 95.2% had injuries caused by the pressure shock wave, 44.6% had burns, and 41.7% were sick from radiation injuries. The sum of these percentages is 171.5%, which indicates the relatively high occurrence of combined injuries. Table 1, a statistic from Hiroshima, shows how much the protection ratios determined the relative frequencies of various types of injuries.[2]

TABLE 1. INCIDENCE OF INJURIES (%) IN HIROSHIMA AREA (MEDICAL PARTIES, TOKYO IMP. UNIV.)

Location / Type of Injuries	Outdoors	Indoors	Shielded by: Wooden Structure	Shielded by: Concrete Structure	Shielded by: Other
Thermal	81.0	19.0	15.0	2.3	1.7
Mechanical	19.6	80.3	66.2	12.3	1.8
Radioactive	46.3	53.6	43.1	8.1	2.4

Current investigations estimate the probability of combined injuries even higher than the figures known from Japan. For example, Geiger, from the DDR (East Germany), writes in his "Foundations of Military Medicine"[3] that in a nuclear war, 65-70% of the injured would suffer from combined injuries. The probabilities of the various combinations would be as follows:

trauma + burns + radiation, up to 20%;
burns + radiation injury, up to 40%;
trauma + radiation injury, up to 5%; and
trauma + burns, up to 5%.

PATHOGENESIS AND CLINICAL MANIFESTATIONS OF COMBINED INJURIES

The physicians in Hiroshima and Nagasaki observed a great aggravation of an injury when combined with radiation. For example, mechanical wounds or burns seemed to heal at first; however, 1 or 2 weeks later, a serious relapse in the healing process could be observed. Wound infections returned with disorders in the granulation formation; a gray, greasy coating would form on the wounds. The necroses, caused by the granulocytopenia, and the collapse of resistance against infections would occur. The thrombocytopenia would cause a renewed occurrence of hemorrhages in the area of the wounds. From a histological viewpoint, wounds of the skin, burns, necroses, and massive bacterial infiltrations could be observed. Collections of leukocytes, collections that would normally limit a dead tissue area, were absent due to the agranulocytosis[4] caused by the whole-body irradiation. This pathogenetic pattern was completely new to the Japanese physicians, who called this unexplainable disease "Gen Baku Sho," that is, atom bomb disease.[5]

Combined injuries caused by both whole-body irradiation and wounds or by burns is still a largely unknown clinical picture. The cause of this is that Japanese and American informants paid attention only to the individual injuries, especially those of subjects with acute radiation diseases that had not been observed until then. In the years thereafter, under the impression of a global nuclear threat, an intensive radiation biological investigation was initiated, especially in the military laboratories in the USA, the European countries, and the Soviet Union; however, the study of combined injuries was relatively small. The cause of this might be that many investigators are of the opinion that combined injuries, are just modified radiation injuries for which the same treatment principles are valid, as compared to radiation diseases.

However, there are authors who consider combined injuries as something special. For example, Federov[6] writes that "the radiation-burning trauma" is a new disease that differs significantly both from irradiation syndrome and from burn diseases. Chromov, Perkutov, and Lubenski[7] posed the basic question of whether for combined injuries the various traumas amplify each other

mutually and make the typical symptoms just stand out better, or whether they lead to a completely different clinical picture. Generally, there is hardly a satisfactory answer to this question, if one considers various traumas, even in combination with the same type of injury each time, i.e., the radiation exposure does not necessarily cause different reactions in the organism. It is certain that the main symptoms of the individual injuries remain present and are only modified. However, it can happen that the symptoms that play a relatively unimportant and hardly recognizable role in various individual injuries become so prominent in combination with other noxae that the entire clinical picture of the new symptomatology is determined by these previously unimportant symptoms, and one is tempted, in such cases, to talk of a "third disease."

We already mentioned that a satisfactory evaluation of the many combined injuries that occurred in Hiroshima and Nagasaki does not exist, and that also no other clinical experience with combined injuries is available that could offer significant evidence with respect to the questions discussed. Therefore, it is necessary to base ourselves on experiments with animals; thus, the following explanations and interpretations are made with respect to experiments with animals.

ANIMAL EXPERIMENTS AS A BASIS FOR PATHOLOGY AND THERAPY OF COMBINED INJURIES

In the combined injuries, burns seem to worsen the prognosis of a whole-body irradiation to a greater degree than other traumas. This is shown by the investigations of Brooks et al.[8], Baxter et al.[9], Alpen and Sheline[10], and Korlof[11], with dogs, pigs, rats, and guinea pigs as subjects. The results, summarized in Table 2, show that an addition of the radiation and burn lethality in the test animals was not often produced but that in these injury combinations, magnification effects could occur. An extreme increase in lethality was observed, especially for the larger test animals such as dogs and pigs, which are more similar with respect to their sensitivity to radiation than rodents.

TABLE 2. COMBINED EFFECT OF WHOLE-BODY IRRADIATION AND APPROXIMATELY SIMULTANEOUS BURNS ON VARIOUS ANIMAL SUBJECTS

	Lethality (%)
Dogs (Brooks, Evans, Ham, and Reid)	
20% burns	17
100 R	0
20% burns + 100 R	73
Pigs (Baxter, Drummond, Stephens-Newsham, Randall)	
10-15% burns	0
400 R	20
10-15% burns + 400 R	90
Rats (Alpen and Sheline)	
31-35% burns	50
250 R	0
500 R	20
31-35% burns + 100 R	65
31-35% burns + 250 R	95
31-35% burns + 500 R	100
Guinea Pigs (Korlof)	
1.5% burns	9
250 R	11
1.5% burns + 250 R	38

Without doubt, open wounds that do not close again form a great danger for the survival of the test animals. In our own investigation we could prove[12] that mice irradiated with an LD_{26} (in this case, 510 R) and inflicted 2 days later with an open skin wound, died in 90% of all cases. If the wounds were covered immediately, the mortality could be decreased down to 18%. However, if the wounds remained opened, the animals soon became very sick. They lost up to 30% of their body weight. After an initial lack of appetite, they drank a greater amount of drinking water because they clearly lost a great amount of liquid through the open skin wounds. The excreted amount of

urine decreased drastically. The consumption of solid food stopped or was interrupted for at least a few days. An increased amount of urea was excreted in the urine, which in its turn caused an increased proteolysis caused by an acidotic condition of the metabolism. The histological picture indicates a protracted shock with definite microcirculatory interferences.

Poljakow[13] confirmed that a shock syndrome can play a decisive role in combined injuries. He writes, "Already during the latent phase of the radiation disease, the shock causes drastic changes in the metabolic processes. The reduction and oxidation processes in the tissues were highly slowed down. . . . At the high point of the radiation disease a completely insignificant mechanical trauma can cause a shock. . . . The arterial blood pressure decreases markedly and can be raised only with difficulty."

The extensive investigations of Mitrofanov[14] into the oxidation and reduction processes in the tissue respiration indicate as well such a synergy between trauma and radiation effects. The dogs he investigated showed, due to the combined injuries, a lowering of the capability to oxidate for the cells in the cerebral cortex in the brain stem, liver, kidneys, heart muscle, and lungs.

Cluzet[15] was the first to report about the effects of X-rays on the healing of fractures. His study and those of other authors addressed themselves to the question of how far a local irradiation delayed the formation of callus and the consolidation of a fracture. However, in combined injuries caused by nuclear explosions, an irradiation of the whole body with a subsequent radiation syndrome is produced. So in this case, rather than local exposure to radiation of the fractured region, the disorders of the entire organism with their side effects on the healing process of the fracture are most important.

Zemljanoj[16] could observe that the formation of callus was significantly delayed in an artificially fractured body of a test animal that had been exposed to whole-body irradiation in addition. Figure 1 is drawn with the aid of X-rays; it shows the course of healing of lower arm fractures in rabbits that have been irradiated with 800 R; 2 hours later a piece of the radius

subperiostal was taken away. In all animals, the picture of a heavy radiation disease developed, which caused the death of 20%. On the 32nd day, the bones of the control group were completely healed, the callus was extensively built up again, and the marrow hole was open. In the irradiated animals, a gaping opening between the bone ends still existed, and only after 60 days was a complete healing of the artificial fracture reached.

These X-ray investigations additionally show that the regeneration of the bone tissue is not interfered with to a great degree during the first days after the irradiation; however, at the maximum of the radiation disease, a very definite retardation of the healing of the fracture can be observed accompanied by disorders in the callus formation and delays in the restoration of the bone marrow hole.

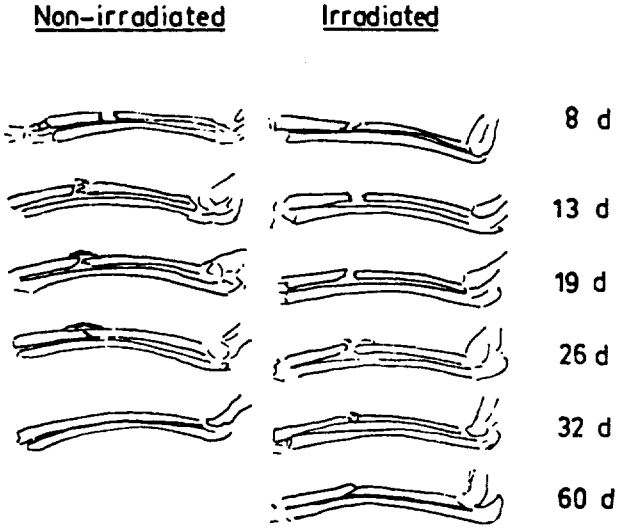


FIGURE 1. Fracture healing (rabbit radius) after whole-body irradiation with 800 R as compared to non-irradiated controls

In nuclear explosions, pressure, heat, and radiation appear simultaneously. Therefore, in most experiments with animals, the irradiation and the second injury were placed together as closely as possible. However, it might be possible that the exposure to radiation and a second and also third trauma are inflicted on the organism at different times. An example of

this is when people have been exposed to a shock wave or thermal radiation and subsequently are exposed to gamma and beta radiation from the fallout. There is also the possibility of a reversed sequential order in the injuries in the following case: subjects are exposed to fallout radiation or initial neutron-gamma irradiation, and are subsequently burned and/or injured by conventional or nuclear weapons. Furthermore, the necessity can arise for exposing an irradiated subject afterwards to the trauma of a surgical intervention. In the following we will present the results of our own investigations into the problem of the time factor.

The first, rather unexpected results were observed in mice whose spleens were removed before or after a whole-body irradiation.[17] If the intervention was performed 2 days before the radiation, the radiation lethality decreased from 27% to 10%. If the splenectomy was performed simultaneously, i.e., 1 hour after the irradiation, the lethality of the animals even decreased down to 4%. However, if the operation was performed 8 days before the irradiation, the radiation lethality did not change. The effects of the splenectomy were different when performed 2 or more days after irradiation. In that case, the lethality increased to values much larger than the radiation lethality. If the spleen was not removed during the abdominal intervention so that only a test laparotomy was performed, the influence of the operation on the radiation lethality was clearly lower. Only when both traumas were inflicted approximately simultaneously was a decrease in the mortality of the mice observed. Sham operations performed 2 or more days before or after the irradiation produced a moderate increase in the lethality.

Instead of the mentioned closed interventions, if open bleeding skin wounds were made, a significant increase in the lethality was produced when performed approximately simultaneously with the irradiation, as can be seen in Figure 2. However, a decrease in the radiation lethality could occur when the intervention was performed a number of days before irradiation; the lethality increased considerably when the wounds were produced after the irradiation. However, if the skin wounds were not left open but were sewn up shortly after the intervention, the radiation lethality would be influenced to a much lower degree. A significant decrease in the lethality was obtained by performing the operation at approximately the same time as the irradiation; a slight

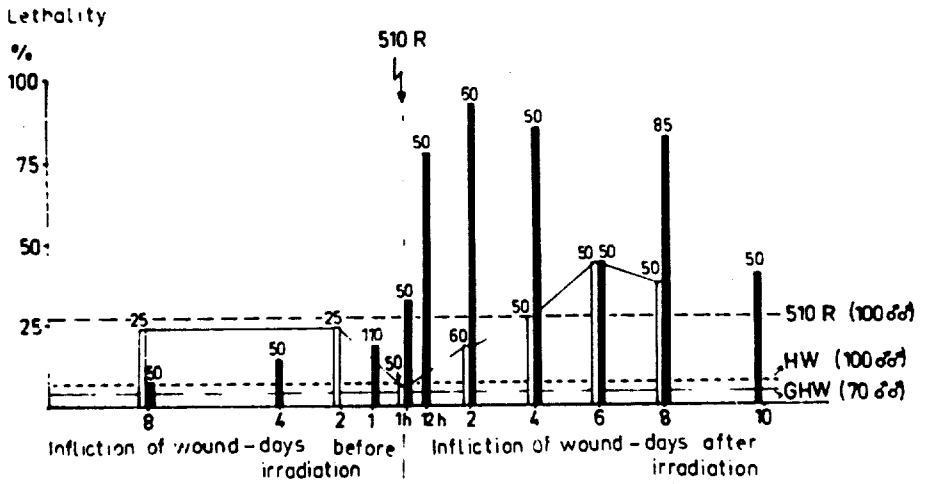


FIGURE 2. Mortality (%) of male mice as a function of the interval between irradiation (510 R) and infliction of wounds (open skin wounds, HW; skin wounds, hatched columns; closed skin wounds, GHW: white columns).
--- (HW) and —— GHW) = wound lethality without additional exposure to irradiation.

increase in the lethality as compared to the radiation lethality could be observed when the intervention was performed 6 days after the irradiation. Figure 3 shows in a graphic representation the lethality ratios of the mentioned four types of injuries that were inflicted on the mice at various times before or after the irradiation. In this figure the following can be noted: (1) irradiation of previous traumas only moderately affected the radiation lethality of mice or produced a significant decrease, (2) traumas that have been inflicted simultaneously with the irradiation mostly produced a decrease in the lethality, (3) traumas inflicted after the irradiation increased the lethality, and (4) serious interventions such as splenectomy and open wounds influenced the radiation lethality more than light injuries.

We performed investigations in which we inflicted burns, compression injuries (tourniquet), aseptic muscle necroses (with formalin), and various kinds of stress, such as overheating, undercooling, and exhaustion (by swimming) at various times before and after irradiation. However, an

explanation of these almost regular relationships does not seem easy. It seems understandable that the infliction of a second trauma after the irradiation, i.e., the effect of a noxa on the organism with radiation disease, produces an increase in the lethality. In the investigated mice, the open skin wounds, burns, and operations led to serious, irreversible shock conditions, which caused the death of the animals. This assumption is justified by biochemical and histological investigations performed by us. Probably no decisive role is played by infections; the bacteriological control groups, at least, do not allow such conclusions. Hemorrhages might have been of influence; these occurred when the animals were operated upon after the 6th day, i.e., in the stage of thrombocytopenia.

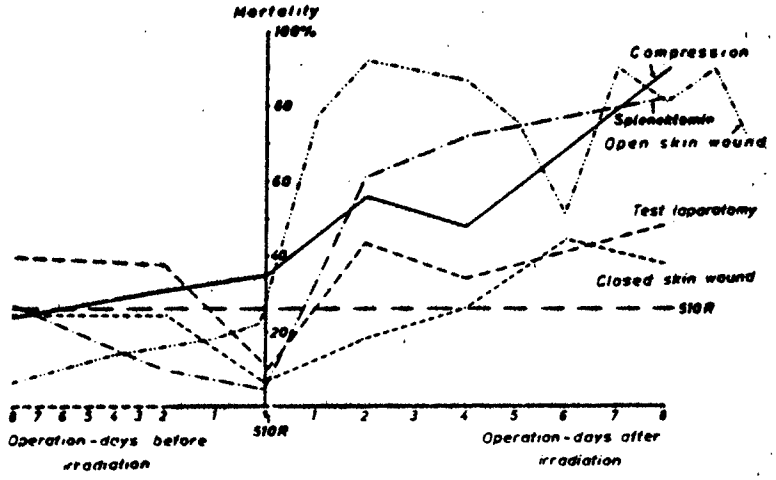


FIGURE 3. Mortality of male mice as a function of the interval between irradiation (510 R) and the performance of additional interventions such as closed skin wounds, spenectomy, test laparotomy, or compression of an extremity (tourniquet)

Significantly more difficult is the explanation for a decrease in the radiation lethality caused by a trauma before or at the same time as the irradiation. A reasonable solution might be to explain the obvious protective effect of preceding traumas by the adaptation syndrome of Selye. That is, we

assume that preceding operations or injuries cause a stress situation that produces an increased ACTH secretion of the hypophysis and a subsequent suprarenal cortex reaction. In our own investigations in mice,[18] we observed a marked increase in the corticoid level of the suprarenal glands after the infliction of open skin wounds; one peak was observed 2 hours after the wounds were caused, and a second peak was observed 2-3 days later. An irradiation that followed a few hours or days after the operation would affect the organism in a condition of an increased suprarenal cortex reaction. Further investigations could demonstrate increased corticoid levels after irradiation as well; the increases occurred at the same intervals and were even more marked. With a reversed order of the traumas, i.e., the wounds were inflicted at a time of an increased corticoid level caused by irritation, no influence on the lethality could be observed. The wounds by themselves were a significantly less serious burden for the organism than was the radiation. In most cases a serious injury accompanied by an increased lethality was produced if the wounds were inflicted after the irradiation.

Further investigations into the "protective effects" of preceding traumas included the investigation of the bone marrow of mice with combined injuries. One can start here with the basic assumption that higher radiation doses produce a higher lethality than do lower doses because a correspondingly greater number of stem cells will be destroyed. If a greater number of stem cells could be observed in the bone marrow of irradiated mice that had been wounded beforehand than in mice that had been irradiated with an equal dose but had not been wounded beforehand, this could indicate an increased radiation resistance in the mice that had been wounded beforehand.

THE THERAPY FOR COMBINED INJURIES

The treatment of combined injuries must always be seen against the background of the radiation syndrome therapy. In addition to this therapy, typical measures for each individual injury have to be applied.

All combined injuries have a set of symptoms in common; general treatment principles can be adjusted to these symptoms. Almost all additional injuries

worsen the development and prognosis of the radiation disease. Radiation doses whose effects are far below the limit of lethality can become life endangering in the presence of serious injuries or burns.

The susceptibility for shock is extremely increased. Not much is known yet about the "combination injury shock" in people; one can assume, however, that its treatment should be similar to that of a wound shock without additional exposure to irradiation. Warmth, quiet, control of pain, and stabilization of the circulatory system should be mentioned here.

The healing of wounds, burns, and open fractures in the initial phase of the radiation disease does not always differ from the healing in non-irradiated patients; however, when the agranulocytosis starts, very serious wound infections can occur, which can even lead to necrosis. The method of choice is a primary wound suturing as soon as possible after the combined injury. Local and general antibiotic therapy should not be omitted.

The increased potential for hemorrhages after irradiation is an additional reason for the requirement that surgical interventions should be performed in the first hours or days after the irradiation. Special attention should be paid to the blood stanching because there is a great risk of after-hemorrhages. Blood transfusions are not indicated very much for "pure" radiation diseases; however, it may become necessary in combined injuries. It is important to note that a surgical treatment, especially in the initial phase, should take precedence over the radiation disease, because the critical symptoms of the radiation disease occur only 1-2 weeks later. If necessary for the injury, antibiotics should be given at an early stage as well, although from the viewpoint of the radiation disease, an antibiotic therapy that precedes the maximum phase does not seem purposeful.

The results of a series of experiments with animals that I conducted are probably of practical significance as well. They show that mice that have been inflicted with whole-body irradiation in combination with skin wounds are changed in their sensitivity to such drugs as Evipan, Luminal, Thiogenal, and

Epontol, as well as morphine, Dilaudid, and Dolantin. This decrease in tolerance could necessitate a greater discretion and a diminishing of the amount of the narcosis substance used.

The recommendations for the treatment of combination injuries discussed up until now differ in many respects from the conventional therapy of open injuries. In particular, the primary wound closure might seem very questionable in the case of an extensive infected and fragmented injury, especially under war conditions. In addition, the danger for an anaerobic infection is increased in the presence of an exposure to irradiation. Figure 4 shows that artificial gas-gangrene infections lead to a higher percentage of deaths when the infection is inflicted at the maximum phase of the radiation disease.

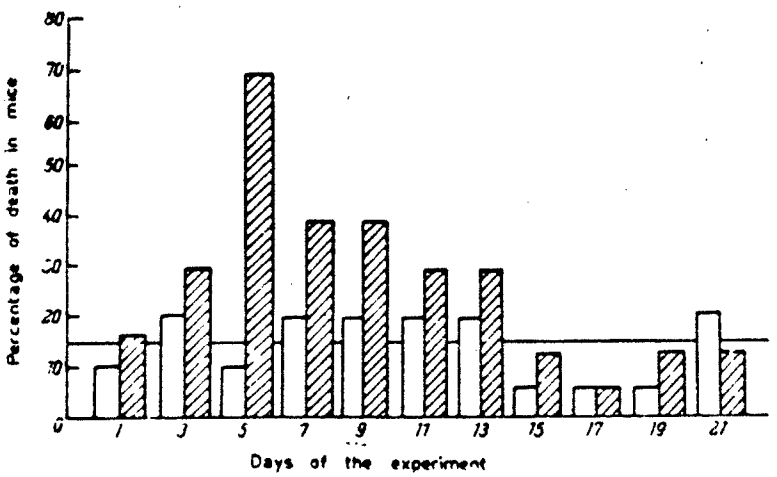


FIGURE 4. Changes in the susceptibility for gas-gangrene infections of irradiated white mice at various time intervals after the irradiation (according to Petrov [19]). White columns: control mice; hatched columns: irradiated mice.

For the planning of therapeutic measures, it is of decisive importance to know whether a significant irradiation really has taken place in addition to the other injuries. In almost all animal experiments, the subjects were exposed to sub-lethal or roughly medium-lethal whole-body irradiations and subsequently inflicted with the various types of injuries discussed. Therefore, the radiation component was always very precisely defined.

However, in a nuclear catastrophe, this doesn't have to be the case at all. Probably there will be a great percentage of injured who have not been exposed, or have been slightly exposed, to irradiation; therefore, these subjects should not be treated like combined injured patients.

The estimation of the degree of radiation damage simply belongs to the greatest diagnostic difficulties. The physical radiation measurement, i.e., the so-called individual dosimetry, is greatly insufficient and perhaps will remain that way forever. Only the close collaboration of the surgeon with a radiologist who is extremely familiar with radiation-biological problems and with a hematologist can lead to success in these difficult decisions. The findings described are essentially based on experiments in animals, and nobody knows how far these are valid for human subjects.

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IMPACT OF IONIZING RADIATION ON RESPONSE TO THERMAL AND SURGICAL TRAUMA

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INTRODUCTION

Triage criteria and therapeutic tactics are relatively well established for polytraumas, even in typical mass-casualty situations. Far more uncertain is the management of patients demonstrating radiocombined injuries. For that reason, we have carried on a research program on irradiated animals with either burns or surgical trauma (partial enterectomy or nephrectomy). The research's aims were (a) to follow the evolution of the clinical and biological syndromes of these pathological entities; (b) to determine anaesthesia, resuscitation, and surgery problems presented by the irradiated animal in toto; and (c) to specify repair mechanisms after combined injury.

Our results represent an original contribution to the study of radiocombined injuries. Radiation and visceral surgery with the large-animal models have already been the subject of research efforts (Tichonin, Kotelnikov, Gorelov, reported by Messerschmidt,[1] Gustafson and Cebus,[2] Piscevic et al.[3]). In other respects, many authors have studied the clinical and biological evolution of burns combined with an irradiation (among them, Alpen and Sheline,[4] Korlof,[5] Vogel,[6] Valeriote and Baker,[7] Messerschmidt et al.[8], Nguyen et al.[9]). The use of early excision-graft in the primate exposed to radiation and thermal burns has not been previously reported.

The results presented here represent a synopsis of restrictedly distributed, recently published reports.[10-12]

MATERIALS AND METHODS

ASSOCIATION IRRADIATION-SURGERY

Animals and Experimental Series

Our study was carried out on 72 male adult pure-bred beagle dogs, from 10 to 12 months of age, with an average weight of 11.5 ± 1.5 kg divided into 18 experimental series (Table 1).

TABLE 1. IRRADIATION AND SURGERY: EXPERIMENTAL GROUPS

CONTROL DOGS

Blood Sampling	Enterectomy	Nephrectomy	Irradiation (day J) 2.5 Gy	3 Gy	3.5 Gy
0'6[a]	0/2	0/4	0/4	2/4	4/4
				J+20[b]	J+19

IRRADIATION (day J) AND ENTEREC'OMISED DOGS

at day J + 4			at day J + 11		
2.5 Gy	3 Gy	3.5 Gy	2.5 Gy	3 Gy	3.5 Gy
1/4	3/4	3/4	1/4	2/4	4/4
J+16	J+15	J+13	J+12	J+17	J+15

IRRADIATED (day J) AND NEPHRECTOMISED DOGS

at day J +4			at day J + 11		
2.5 Gy	3 Gy	3.5 Gy	2.5 Gy	3 Gy	3.5 Gy
2[c]/4	3/4	4/4	1/4	2/4	4/4
J+11	J+14	J+19	J+24	J+17	J+16

[a] lethality and number of animals in each group
[b] mean date of death
[c] one in early post-operative period (J+5)

Irradiation

The dogs without anaesthestic and fasting were placed in an Altuglass cage (50 x 40 x 15 cm) located 2 m from a ^{60}Co source. The radiation was delivered in two equal fractions bilaterally. Dose rate was 14.3 cGy/min corrected. The animals were irradiated at 2.5, 3 or 3.5 Gy. We called J the day of irradiation, and the surgical operation took place on J + 4 or J + 11.

The Surgical Intervention

One hour after a pre-operative treatment [2 mg/kg of chlorhydrate pethidine (Dolosal, Specia) and 2 mg/kg or chlorhydrate phenothiazine (Diparcol, Specia).] the anaesthesia was induced by a 25 mg/kg sodium penthothal (Penthothal, Abbott) I.V. injection. An endotracheal tube was linked to a Vapor-Draeger device utilized in half-closed circuit. Gaseous anaesthetic (75% nitrogen protoxyde, 25% oxygen) was delivered at a 1-l/min constant flow. During the operation, we used a combined anaesthetic (Penothal-nitrogen protoxyde) so that the total quantity of injected Penthothal was around 500 and 800 mg. During the surgical operation, the animal received 250 to 300 ml of an isotonic glucose infusion.

The enterectomy involved the excision of 8 to 10 cm of small bowel. The entero-anastomosis was carried out in two planes using interrupted sutures. One million units of Colimycine were placed into the abdominal cavity before closing the bowel in three planes.

The left nephrectomy was performed by a lumbar approach and followed by the deposit of one million units of Colimycine in the renal bed. After the placement of drains (left in place for 4 days), muscular masses were sutured in one plane.

Daily antibiotic therapy with Bipenicillin (500,000 U.) and Colimycine (500,000 U.) was carried out for 5 days. The same treatment was given to unoperated irradiated animals. Gradual feeding was spread over 6 days.

Clinical, Biological, and Anatomicopathological Controls

Animals were clinically examined daily for 2½ months. Blood samples (23 ml) were withdrawn for examination of bacteriology, hematology, hemostasis, and biochemistry.

Necropsies were carried out either after death or animal's sacrifice, 6 months after the radiocombined injury.

ASSOCIATION IRRADIATION-BURN

Animals and Experimental Series

Macaca fascicularis monkeys, with a 5.7 ± 1.2 kg average weight, were divided into six experimental groups (Table 2).

TABLE 2. IRRADIATION AND BURNS: EXPERIMENTAL GROUPS

Blood Sampling	Burn (12.15%)	Irradiation (2 Gy)	Irradiation + Burn	Burn + early excision	Irradiation + burn + early excision graft
0[a]/4[b]	0/3	0/2	0/3	0/5	1[c]/6

a: dead monkey
b: number of animals in each group
c: animal without preventive antibiotherapy

Irradiation

The monkeys were anaesthetized with ketamine (Ketalar, Parke Davis), placed in a restraining chair, and irradiated by a ^{60}Co source at a dose of 2 Gy at a rate of 5.5 cGy/min.

Burn

Burns were administered within 1 hour of irradiation under ketamine anaesthesia. The surface to be burned (12 to 15% of the body surface) was accurately marked off on the animal's shaven dorsal side. Burns were then administered by four 1000-Watt flashlights' lumino-thermal effect for a period of 8 sec. This techniques produces a third degree reproducible, well-limited burn, whose degree and homogeneity have been controlled both macro- and microscopically. The animal was then kept in a warm room (> 22°C) and overfed.

Early Excision-Graft

One day after the burn, under ketamine anaesthesia, 0.04 mm thick skin grafts were cut off the animal's ventral side. After total exeresis of the burned tissues and a filet expansion of the skin grafts, these last grafts were sutured by separated stitches onto the burned area. The wound was then covered by a bandage and the animal's trunk protected by a linen cloth. During the operation each monkey received subcutaneously 30 ml of isotonic solution (glucose, sodium chloride, v/v).

Daily preventive antibiotherapy with 100 mg/kg Cephalotine (Glaxo) and 4 mg/kg Gentalline (Unilabo) was given for 8 days from the day of aggression (irradiation or burn) to all the animals.

Clinical, Biological, and Anatomicopathological Controls

Animals were routinely examined for weight and temperature as well as hematological and biochemical (protidemia, ionogram) parameters.

Skin samplings were made on the same time schedule as the biological assessments.

SYNTHESIS OF RESULTS

ASSOCIATION IRRADIATION-SURGERY

Around Day 10 postirradiation, animals begin demonstrating a state of general impairment. Around Day 12, the dogs become hyperthermic and often showed positive hemocultures. Septicemia was evident in those that died (50%). In those that survived an improvement of the general state was shown from Day 19, and biological normalization was seen around the 6th week postirradiation. Surgical interventions were noted to disturb the pattern of normalization.

Hematological Parameters

Nephrectomy carried out 4 days postirradiation worsened the erythrocyte, leukocyte, granulocyte, and lymphocyte counts more than did enterectomy.

This observation was true for erythrocytes whatever the irradiation dose, and was especially true for leukocytes at doses above 2.5 Gy. If the surgery occurred 11 days postirradiation, it appears that there was no difference between nephrectomy and enterectomy.

Hemostasis

Generally, both types of surgical operations increased the blood coagulation disorders due to irradiation in the same proportions. Specifically affected were platelet count, skin bleeding time, recalcification time (Howell's method), the prothrombin time (Quick's method), the P.P.S. value (factors II, VII, X), the thrombin time, the fibrinogen level, and the thrombelastogram.

In some experimental conditions such as nephrectomy carried out on 11 days after 2.5 Gy irradiation, a few components of the radio-induced hypocoagulability were not increased ("r + k" and "a" parameters of thrombelastogram, platelet count) or seemed to be "improved" ("a" parameter of thrombelastogram, fibrinogen level) (Figure 1).

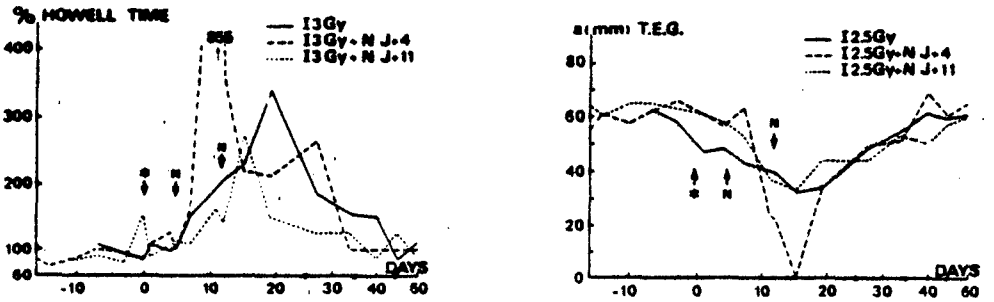


Figure 1. Changes in recalcification time (Howell) and "a" parameter of thrombelastogram (T.E.G.) with dogs after whole-body irradiation (*;I) alone and after nephrectomy (N) 4 days (J+4) or 11 days (J+11) post irradiation.

Serum Biochemical Syndrome

Electrolytes (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, P)

In our experiments, radiation appeared to modify potassium, calcium, and chlorine levels.

Enterectomy added to irradiation only slightly worsened this imbalance (calcium, potassium), whatever the surgery schedule. The irradiation-nephrectomy association more severely disturbed the ionogram (sodium, magnesium, mineral phosphore) but only when the surgery was performed 4 days postirradiation.

Proteins and Proteinogram

Between 4 and 24 hours, some irradiated animals showed a transitory hyperprotidemia or an hypoalbuminemia.

A partial ileectomy associated with an irradiation led to a moderate hypoprotidemia with dogs operated 4 days after 2.5 and 3 Gy irradiation. Nephrectomy caused severe proteinemic deficiency. Radiation-nephrectomy worsened the serum proteinic imbalance of the acute irradiation syndrome in the form of hypoalbuminemia with α_2-and β-hyperglobulinemia. The imbalance was independent of time.

Serum Lipids and Lipoproteins

Irradiation with 2.5, 3, or 3.5 Gy for 11 days induced an increase in the various serum lipidic components (total lipids, cholesterol, triglycerides, unesterified fatty acids) and a decrease in the heavy/light lipoproteins ratio. Nephrectomy further increased this lipidic imbalance.

Serum Enzymes

We have studied 9 serum enzymes: pseudo-cholinesterases, creatine kinase and its isoenzymes MM and MB, lactate dehydrogenase and its isoenzyme 1 and 2 (α-hydroxybutyrate dehydrogenase), 5'-nucleotidase, ornithine carbamyl transferase and alkaline phosphatase.

The irradiation-surgery association diversely affects these parameters according to the studied enzyme, the irradiation dose and the time between both aggressions. Radiation seems to affect the hepato-biliary system (pseudocholinesterase, alkaline phosphatase) to a greater degree than the myocardium (lactate dehydrogenase). With certain exceptions (e.g. 5'-nucleotidase) greater disorders are noted when nephrectomy is done 4 days rather than 11 days postirradiation.

Anatomicopathological Observations

Macroscopic and microscopic injuries (hematopoietic system, lungs, digestive tract) appeared to be the same in irradiated or irradiated-operated animals, and they were generally proportional to the radiation dose. Extensive diffuse parenchymal congestion was observed with the polytrauma animals. In other respects, the surgery increased the infectious risk of irradiated animals reflected by increased frequency of microbial concentration centers in histologic sections of the hematopoietic system, lungs, liver, and kidney.

Healing Intestinal Area

Two weeks after surgery, the entero-anastomosis carried out on the irradiated animals were all tight and functional, but were far more congestive than on non-irradiated animals. Entero-anastomosis areas examined 6 months after the combined injuries showed satisfactory although delayed healing processes. The delay seemed to be due to the qualitative and quantitative deficiency of the collagen synthesis.

IRRADIATION AND BURN FOLLOWED BY AN EARLY EXCISION GRAFT

Clinical and Biologic Parameters

No lethality was observed with irradiated, irradiated-burned, grafted or non-grafted animals, when those modalities were followed by antibiotic therapy. Moreover, the irradiation and the excision-graft did not largely modify the temporal evolution of animals subjected or not subjected to a burn. Contrary to hematological parameters (erythrocytes, leukocytes), the biochemical alterations due to the burn-excision-graft were not increased in the 2-Gy irradiated animal (ionogram, proteinemia).

Skin Repair Phenomena

The Burn's Macroscopical Evolution

Irradiation did not lead to any appreciable modification in the early healing processes of burns. Around the tenth day or so, the impaired, rigid skin began to dissociate, and by month's end, the underlying granulation tissue was readily apparent.

Later, signs of skin healing (regression of the red-purplish-blue color of the granulation tissue, centripal epidermization of the wound and progressive reduction) were seen in irradiation monkeys. Healing was not completed on J + 105 with irradiated-burned animals, whereas burned-only animals demonstrated a glabrous and pink scar by day J + 91.

The Grafts' Evolution

Macroscopically, no difference was observed in the evolution of burns between irradiated or non-irradiated animals. The re-epidermization of the grafted area was complete by the end of the second week. On the contrary, histological observations showed a slight delay in the graft's healing processes in the irradiated animals. This fact was true for the graft (light epidermal proliferation, scar dermal conjectivo-vascular neoformations) as well as for the granulation tissue (delay in maturation).

DISCUSSION AND CONCLUSION

In our experiments with canines previously irradiated *in toto*, the prognosis postileectomy or nephrectomy varied directly with the radiation dose. Contrary to reports by Gustafson,[2] we have found surgery to affect the prognosis only slightly. Messerschmidt *et al.*[3] found similar results when examining stitched skin wounds in irradiated animals. Earlier lethality noted with irradiated-operated animals was apparently linked to infectious pathology, with pulmonary predominance, and which was responsible for a quick cardio-respiratory decompensation.

No clear distinction can be made between radiation and time of surgery and subsequent prognosis. Nevertheless, large systems of regulation succeed in rather efficiently correcting some biological disorders induced by the radiocombined injury, hypoprotinemia. When a delayed surgery (J + 11) disturbs the serum biochemical syndrome, intensity of the observed modifications has never been higher than the one noted after a nephrectomy of J + 4, and has never shown a dose-effect relationship.

The surgery and anaesthetic technique does not encounter any major technical difficulties. In case of acute hypocoagulability, satisfactory hemostasis in the surgery field is possible, and must be promoted by correction of the hypocoagulability syndrome. In that respect, hypocoagulability does not necessarily call for a fatal evolution.

Entero-anastomosis, or kidney healing, follows a satisfying long-term course in spite of the delay noted in the healing processes of the irradiated animal. Stitching rupture or wound dehiscence are not often noted.

The above leads us to assert that whole-body irradiation around an LD 50/30 dose does not constitute a major contra-indication for intestinal or retroperitoneal surgery, even if it must be carried out at the end of the latent period of the acute radiation illness. The increased infectious risk with granulopenic or lymphocytopenic subjects must be considered.

Conclusions were similar for the study of a primate with a third-degree burn on 15% of the body surface effected 1 hour after 2 Gy of radiation.

Radiation delayed the healing processes for 2 weeks, but did not diminish the prognosis of the burn. This runs counter to the observations of Brooks et al.,[14] Baxter et al.,[15] Alpen and Sheline[4] and Korlof,[5] but agrees with the observations of Messerschmidt et al.[8] and Nguyen et al.[9] Finally, early excision-graft, strongly advised in the treatment of the burn, is still recommended in irradiated-burn subjects. The evolution of the early excision graft is minimally influenced by irradiation, which minimized infectious and metabolic problems.

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DISCUSSION PERIOD WITH DR. DUBOS

DR. HIRSCH: Dr. Dubos, in you burn model if you would have chosen a smaller mesh in your graft, would the healing process have been faster? Also, what happened to the donor surfaces of your animal and what was the healing period of your donor surface?

DR. DUBOS: I shall begin with your second question.

Donor surfaces repair in normal time. Healing processes are not disturbed by the radiation. Donor surfaces were vulnerable for monkeys and grafts were not done on those surfaces. Healing processes appeared correct.

We have not tried other dimensions of mesh. Our methodology essentially followed the Japanese work. They tried several sizes of mesh expansion and we have used their dimensions.

The normal skin was two millimeters. The net had two millimeters of large normal skin and it was the better condition.

DR. BAINES: I would just like to point out two things. One, we have shown previously that kinetics of protein synthesis, collagen synthesis in particular, are altered in burned animals versus control animals. even in control sites, within an animal that has received a 20 percent full-thickness burn. In an animal that has a 20 percent full-thickness burn and then has trephine punches removed from the burn site, viable tissue does grow underneath the eschar. In a control site at least two to three centimeters away from the major burn trauma, you find healing with respect to collagen synthesis normal.

In tissue that is coming in, surrounded by eschar, collagen synthesis is delayed at least five to six days, primary peak. Normally in skin you will see a synthesis day four, day five. Well, that peak is delayed at least a week and is diminished. So that looking on days four and 11 is not going to show you what is happening in protein synthesis.

Secondly, histological examination alone is not going to tell you what is going on in a wound. You must also do specific stains for specific proteins. Collagen synthesis, non-collagen protein synthesis, and total protein profiles may be quite different whereas the histology may not be.

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MULTIPLE ORGAN SYSTEM TRAUMA: THE REGIONAL TRAUMA CENTER APPROACH

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INTRODUCTION

Many of the principles and methods currently employed in the care of trauma patients were developed in the military confrontations of this century. The urban centers and highways of the modern era themselves represent battlefields. Trauma is the leading cause of death for Americans between the ages of 1 and 39. It kills over 100,000 people annually and disables another 400,000. The national cost of this problem is enormous, totaling more than $88.5 billion in 1980 alone.

This paper will review the modern concepts and systems approach to the management of multiple organ systems trauma. This type of trauma usually results from blunt impacts, such as those associated with vehicular accidents and with falls from great heights. This type of trauma has a higher mortality than that associated with penetrating injuries.

Published experience of Emergency Medical Services-Trauma Systems[1-5] documents the advantages of the development of statewide Emergency Medical Services programs and regional trauma centers to deal with this problem. Direct transfer of injured patients from the scene of the accident to a distant trauma center was a concept developed during the Vietnam War. In the last 10 years, the regional trauma center model has been further evolved in a number of areas of the country. The experience of these units has underscored the importance of highly organized, aggressive management in the acute phase of polytrauma care. Patients must be treated definitively within the "Golden Hour" following injury, if shock and death are to be avoided. The prime lesson here is that the proper logistics and coordination of multi-specialty care are essential for the optimization of results in the treatment of the multiply injured.

Systems analysis of the modern regional approach to blunt trauma care is relevant in the context of this conference not only as a review of techniques in trauma management, but also as a model for the organization and delivery of care. This may be useful in planning of the possibility of caring for large numbers of patients with combined radiation injury and trauma. If appropriate, I will comment directly on the relative correlaries of the military medical organization.

PATIENT SELECTION AND TRIAGE

When considering a regionalized system it is neither appropriate nor cost-effective to transfer all trauma patients to a regional center. Only the most severely injured class of patients should be transported to these centers. To optimize the care of these patients, they should be identified as being in that class at the scene of the accident, and be transported directly to the trauma center. This obviates the need for screening patients at the nearest medical facility. The direct field triage to trauma center requires that patient selection be made by the paramedical field personnel. This includes Emergency Medical Technicians, paramedics, and police. The Maryland Institute of Emergency Medical Systems has developed a set of criteria used in its statewide system to select patients for transport to the central trauma center. This includes the following: significant head injury, significant spine injury, injury to two major body cavities, combination of injury to a major body cavity and long bone fracture, multiple long bone fracture, complex open fractures.

The effective use of such a set of criteria by these personnel in the field requires constant reinforcement through an extensive ongoing educational program. In the Maryland experience, only 10% of patients triaged are inappropriate for the trauma center.

Time for entry into the system could be further shortened by the use of the central emergency telephone number 911. This enables passersby to more easily contact and bring the paramedical field personnel to the scene. A

well-developed radio communications system is also necessary to coordinate transport of the patients from the accident scene to the central trauma unit. Paramedics with field experience can be used most effectively as the dispatchers in the central communications center. These people speak the language of the field personnel and provide an interface that encourages the use of this system. Voluntary systems, such as the Life Flight program in Houston, Texas, depend on the positive attitude of the field personnel for contacts. The legislatively mandated triage like that in Maryland requires the cooperation of EMT's and paramedics to achieve full compliance.

FIELD TREATMENT

Although it is universally agreed that greater levels of training and experience of field personnel is beneficial, advanced training can sometimes have a negative impact. Well-intentioned paramedics all too often can spend excessive time at the scene of the accident while attempting to insert an endotracheal tube and start intravenous lines. The patient's chances of survival are directly related to the speed with which he is moved to the definitive-care facility for surgical treatment and blood replacement. Many authorities favor the "scoop and run" concept, since it is felt that intubation and intravenous therapy should be accomplished en route to the trauma center, rather than delaying transport. Although the technique of endotracheal intubation is more difficult to learn, once mastered, it provides more secure control of the airway than the esophageal obturator. Packaging should emphasize stabilization and protection of spine, pelvic, and femoral fractures. Excessive time spent in splinting peripheral skeletal injuries should be discouraged in polytrauma patients. External pneumatic compression and auto-transfusion provided by military anti-shock trousers is promising, but their efficacy has not been established in large, well-controlled, randomized studies.

Hospital-based helicopter systems such as the Houston Life Flight program have explored the possibility of carrying nurses and doctors on flights. This enhances the possibilities for early diagnosis and therapy. In many cases, however, the hurried pace and lack of hospital equipment prevent the doctor

from providing care that is any more sophisticated than that offered by a paramedic. A flow of information about the patient can be initiated by in-flight communication to the receiving facility. In view of the high noise level associated with helicopter operation, special monitoring equipment is necessary to take in-flight blood pressures. Telemetry equipment, which is helpful with medical emergencies, has not proven cost-effective with polytrauma patients.

The concepts of paramedic triage and helicopter transport have, of course, developed in the military. Many of the paramedics and medical evacuation helicopter pilots in the civilian sector were in fact trained in the military during the Vietnam conflict. Future confrontation involving the use of tactical nuclear weapons could be associated with a pattern of injuries bearing greater similarity to multiple-organ-system injuries seen in blunt civilian trauma. Dealing with a large number of such injuries will require highly organized patient selection and triage. The complexity of the problems associated with this multi-system trauma have increased the demands on the military care system, both in theater and back at CONUS. This eventuality is being addressed in part, in the development of the Civilian-Military Contingency Hospital System. Civilian trauma center experience suggests that great care must be taken to catalog the exact resources and staffing potential of all institutions involved. Irving Fell, M.D., in Michigan, has created what is considered to be the most thorough and comprehensive catalog of burn beds available in the United States. This type of information should certainly be considered in developing an evacuation plan for combined radiation and trauma injuries. Extensive preplanning would be necessary to properly organize the care of large numbers of multiply injured soldiers during the various phases of their care.

TRANSPORTATION

Successful treatment of multiply injured patients with evolving hemorrhagic shock requires rapid transport to the definitive care facility. The value of helicopters in this effort was clearly established in Vietnam. The technology has been developed in many areas of the United States, and has

shown to be particularly valuable in the movement of victims of vehicular accidents on our highways. As mentioned above, the deployment of helicopters must be coordinated by central communications centers. Several models for helicopter use have been established. The oldest system, in Maryland, employs state-police helicopters, which are stationed at bases scattered around the state. The medical evacuation function now represents 70% of their flight hours. Their ships are Bell Jet Rangers and Hueys. They have a two-man state-police crew, comprised of a pilot and an advanced EMT-paramedic. Placing the helicopters at a distance from the hospital precludes routine flights by doctors and nurses to the scene, but they can shorten the time between helicopter dispatch and patient arrival at the trauma center.

The hospital-based helicopter system is represented by the Life Flight program at Hermann Hospital in Houston, Texas. They have a group of three Twin A Star helicopters stationed at the heliport immediately outside the Emergency Center. The crew in this case includes the pilot, a flight nurse, and a surgical resident. This program also employs a Lear jet and a Navaho fixed-wing aircraft for long-distance medical evacuation. In the case of both of the programs mentioned, the communication center is used to alert the trauma admitting area of the expected arrival time and the condition of incoming patients. Information on these patients can be updated during the flight. Such communication is essential to create a state of maximum readiness for immediate treatment of the patient on arrival.

The military overseas medical evacuation in the modern era is generally accomplished with the use of C-140 transport aircraft that have been adapted with the use of special conversion kits for this purpose. Consideration will have to be given to possible modification of these conversion kits to facilitate the accommodation of multiply injured patients with combined radiation and trauma. This includes the necessary equipment for ventilatory support and physiologic monitoring. Due to the complexity of this equipment, a limited number of aircraft would have to be permanently set up for the function of transporting the more seriously injured.

Massive overseas evacuation requiring activation of the Civilian-Military Contingency Hospital System would necessitate the use of a central administrative triage system. Efficient and safe placement of a large number of patients by this system would require constant communication regarding the availability of various types of hospital beds.

RESUSCITATION AND EVALUATION

The first few hours of activity that occur once the patient reaches the trauma center are extremely critical. During this period of time, resuscitation and evaluation are accomplished. The majority of multiple-organ-system injuries are associated with evolving hemorrhagic shock. Resuscitation and evaluation of these patients become a race, wherein blood and fluid replacement and diagnosis of major bleeding points must be done fast enough to avert the development of irreversible shock. This mission can be accomplished only if an adequate number of experienced personnel are present in readiness upon arrival of the patient. Each member of the team must have specific duties for proceeding in an orderly and efficient manner. They should operate according to predetermined protocols that incorporate goals for the resuscitation.

Following endotracheal intubation, aggressive ventilatory support should be instituted, utilizing volume cycle control positive-pressure ventilators with associated positive end expiratory pressure. Such aggressive prophylactic ventilatory support can help prevent the worsening of the post-traumatic respiratory derangement. Additional intravenous lines should be started to permit more rapid fluid infusion. The choice of crystalloid or colloid fluid for resuscitation is still controversial. It appears certain though, that increased capillary permeability associated with a shock state will allow the large high-molecular-weight protein molecules of colloid to pass into the interstium. In cases of severe hemorrhage, life-saving blood transfusions may be necessary prior to the availability of properly cross-matched blood. The University of Maryland Shock Trauma Center addressed this eventuality by installing a blood bank refrigerator in the admitting area, which is stocked with units of O-negative uncross-matched blood. Patients

presenting with hypovolemic asystole should have an immediate emergency thoracotomy in the admitting area, to permit direct cardiac massage and cross clamping of the supradiaphragmatic aorta. Use of naso-gastric drainage, Foley catheterization, and EKG monitoring should be accomplished at this time.

The first priorities for evaluation and treatment are stabilization of the cardiovascular, pulmonary, and neurologic systems. Following this, careful systematic review of all body areas is conducted. The cervical spine in all of these patients must be protected with a semi-rigid orthosis like the philadelphia collar. All patients are considered to have cervical spine injuries until proven otherwise. The collar is kept on until this proof is obtained, which in some cases may take several days before the C-7 T-1 junction is visualized. Protocol for radiographic examination is included in the following order: lateral cervical spine view, upright chest X-ray (in the absence of any indications of spine injury), and AP view of the pelvis. These studies are the most valuable screening X-rays, and should be performed in exactly that order. At this point, a thorough neurologic examination should be conducted, and injured extremities should be splinted and elevated. The combative patient must be restrained so that he does not pull out all his tubes. It must be remembered, however, that hypoxia is a frequent cause of restlessness.

Forty-percent of polytrauma patients seen in large blunt trauma centers have significant head injuries. Early computerized axial tomography is essential to the discovery of extracranial mass lesions and their early surgical evacuation. If cerebral contusion is diagnosed, the management of the patient will be facilitated by the insertion of an intracranial pressure-monitoring device. This procedure must be done in the operating room. The intraventricular catheter or Richmond screw can be inserted as an initial procedure. The presence of this device for continuous intracranial pressure monitoring will facilitate long hours of general anesthesia, during which neurologic examination can be completed. Steroids, hyperventilation, and appropriate fluid therapy can be used to reduce cerebral swelling.

Diagnosis of intra-abdominal injury by classic techniques of physical examination is difficult and unreliable in this setting. Concurrent head and chest injuries, associated drugs and alcohol, and the limitations of time all lead to errors in diagnosis and to unnecessary mortality, if these patients are treated like those with abdominal pain. The mini-laparotomy and lavage technique substantially improves diagnostic capability for intra-abdominal bleeding. This procedure can be performed in the admitting area. After directly exposing the peritoneum through a small incision, a peritoneal dialysis catheter is directly inserted. A liter of lactated Ringers is infused and then evacuated. The effluent can be checked for color, cell counts, and enzyme levels. This is a remarkably sensitive technique, and even small amounts of blood can be detected, making the incidence of false negative taps very low. On occasion, blood from a retroperitoneal hematoma will leak into the abdominal cavity, causing a false positive tap. This occurs particularly in the face of major pelvic fractures. The most difficult injuries to control for the abdominal surgeon are a burst liver injury and a massive pelvic fracture. These are highly vascular areas with diffuse circulation, and they present major challenges for hemostasis. Most other injuries can be controlled by repair or removal.

Angiography is an extremely important aid to diagnosis and treatment. Patients with widening of the mediastinum on the upright chest X-ray should undergo aortic arch angiography. During this angiography, it is easy to shoot the celiac axis and pelvic vessels as well. With the advent of digital angiography, angiographic data can be obtained in a much shorter period of time. In many instances, the surgeon can be provided with a road map of the bleeding before he enters the abdomen. Angiography becomes therapeutic in the case of pelvic fractures, where selective catheterization can be used to embolize bleeding arterials with autologous clot, gel foam, and metal coils. Arteriography has not been found to be reasonable on a routine basis for major pelvic injuries. Only those pelvic fractures that show continued demand for transfusion after stabilization by external fixation and fluid replacement should be considered for this modality.

MULTI-SPECIALTY SURGICAL CARE

Treatment of spinal injuries will require the collaboration of neurosurgeons, orthopedists, and physiatrists. Unstable spinal injuries should be treated by early spinal realignment and stabilization. Decompression of the spinal elements is better achieved by this method than by laminectomy. Spinal stabilization provides the best medium for recovery of injured and edematous neural tissue. Adequate stabilization often requires internal fixation. Special techniques and instrumentation have been developed to accomplish this mission. The results of studies at The University of Maryland suggest that completion of this surgery within the first 9 hours following injury substantially improves the extent of neurologic recovery.[6,7]

Maxillofacial injuries occur frequently and many groups have an interest in this part of the anatomy. So the division of responsibility for care of these injuries often becomes a major political and administrative exercise.

Vascular injuries require the cooperation of the vascular surgeon and orthopedist. In cases of complete ischemia, the angiography, vascular repair, and skeletal stabilization must be performed expeditiously in order to preserve the limb. Major open fracture with loss of soft tissue and bone is a frequent occurrence in these patients. In recent years, the technique of external fixation has been the object of renewed interest and investigation. Many centers have shown that the treatment of these major open injuries can be substantially improved by the use of primary external fixation, serial debridement, and staged tissue reconstruction. There was a bad experience in the military during World War II with the external fixation systems available at that time. Since then, there have been substantial improvements in the design of external fixation systems and growth in the understanding of their use. The military would do well to consider reincorporation of this technique into its surgical armamentarium.

Primary care of the multiple-system-injury patient following initial definitive surgical treatment generally requires the cooperation of several groups. The general surgeon-traumatologist has primary responsibility for the

patient's care. A growing legion of intensivists, usually anesthesiologists, is now ready to share this responsibility by offering expertise, particularly in the area of ventilatory support. Critical-care nurses assume an important function during this phase. They are with the patients constantly, and develop a close attachment to the patients. Their involvement goes beyond observation and nursing treatment. They become advocates and coordinators, and act as an interface between the patient and his many medical specialists.

Many trauma centers today are struggling to develop systems of computerized documentation and data collection for multi-system injuries, and the subsequent analysis of clinical information could be sustantially aided by computer technology. The medical establishment is far behind the business and military spheres in its utilization of computers for information handling. The development of the injury severity score has given us a means to compare the results of various centers.[8,9] We have learned much from the experience of previous wars. The military, which is in a better position to adopt computerization, should consider adopting the injury classification schemes and the severity score system utilized for civilian trauma.

Selection and transportation of seriously injured patients to a designated trauma center with necessary resources, staffing, and experience in the treatment of polytrauma, can improve the overall care of these patients. Many lives that were previously lost in the field and in the hospital are now being saved by the utilization of these methods. Unfortunately, in some of these cases, patients will survive only for a few weeks and then will succumb to sepsis and multiple-organ failure. Continuing research will be necessary in the areas of host defense and cellular metabolism following trauma to solve these problems.

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DISCUSSION PERIOD WITH DR. BROWNER

DR. WILLIAMS: We have four trauma centers in Massachusetts and it is from that base that I make these observations. First we have to be careful of some of our generalities. I, haveing spent considerable time with the military, and agree that we have learned a great deal about trauma from military experiences, but I would remind us that at the end of World War II we were absolutely certain that packing liver wounds was a disgrace. It took us 30 years to learn that that was incorrect information.

And, secondly, although I believe in them, you have to remember that in two of the studies patients were picked up and eventually died with ruptured spleens. That requires only a knowledgeable surgeon, not a trauma center.

Those are my observations; now three questions. You said early that the people learn at the site of the accident to judge severity so they can triage. I would challenge that. I think the data on classifications,

Champion's Index and all of them are very secure. You can judge outcome beginning at the scene. More importantly you can, from a classification point of view, judge the severity of the injury by using paramedics and EMT at the scene.

Don't you really believe that one of the advantages of regional systems is the classification so that we compare patients one from another?

I believe in trauma centers, but we must be concerned as to whether trauma centers really have improved outcome.

If you look at the post emergency room patients leaving the hospital, you have substantial trouble proving that any trauma center has made a significant impact on outcome. On this there is almost no data and I think we had better be careful of our presumptions regarding trauma centers.

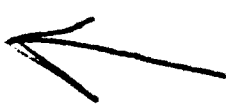
Len Jacobs, has shown that if paramedics treat trauma at the scene you can improve the classification of the injured patient to an improved level from which we presume better outcome would occur but this has not yet been shown.

Patients who don't bleed to death from trauma, may later die of sepsis. Possibly the thing we should be looking at in combined injury, as well as trauma, is what happens systemically to the patient within hours of their injury so that we may predict outcome. Is that not also possibly one of the advantages of trauma centers?

So I believe in them, but I think before we sell the world on them we had better be a lot more self-critical.

DR. BROWNER: I agree with you that we have to go further in terms of proving the effectiveness of regionalized systems. But I think that there are already in existence a number of studies, by Trunkey and his associates out in California, where they compared the San Francisco General system to Orange County before it became organized as a Level II trauma center. The cases that died were the ones where things like ruptured spleens were missed.

Unfortunately, in many systems, where you just pick the patient up and take him to the nearest Emergency Room, things like that happen. They miss things that are terribly simple, like ruptured spleens because many of these hospitals are not used to dealing with poly-trauma patients. Physicians have to be called in from some distance and problems like that can occur.



AD-P003 861

EFFECT OF RADIATION AND SURGICAL TRAUMA ON GASTROINTESTINAL FUNCTION

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INTRODUCTION

The response of the gastrointestinal tract to physical and psychological aggression is initially characterized by an inhibition of its motor and secretory activity. The subsequent response is highly variable and depends on the type and intensity of the stimulus. In the present review, we will consider only the effect of ionizing radiation and of surgical trauma on gastrointestinal motility and secretion.

I. EFFECT OF RADIATION

Radiation sickness is characterized by an array of symptoms that are produced by the action of ionizing radiations[1] (Figure 1).

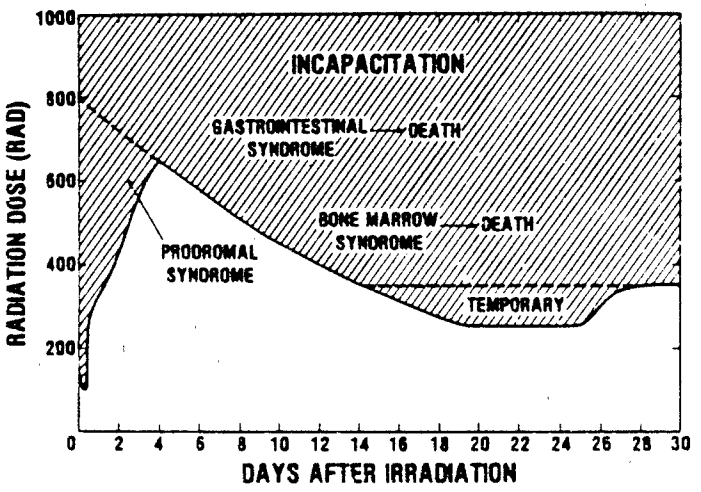


Fig 1 - Schematic two-dimensional representation of incapacitation (hatched area) compared to absence of symptoms (clear area) as a function of radiation dose and time. (After G.A. Grant, A.R. Cairnie, P.K. Harding, N.T. Gridgeman, W.D. Rider: A predictive study of the incidence of vomiting in irradiated military personnel. DREO Report 817, October 1979.

During the first hours that follow exposure, a prodromal syndrome characterized by nausea and vomiting is commonly observed. These symptoms can occur after total body exposure of 1 Gy or more, and usually disappear after a few hours.[2] The pathophysiology of this syndrome as well as the concurrent motor activity of the stomach and small intestine are largely unknown.

After exposure to low doses of radiation, the prodromal syndrome is followed by a remission which may be permanent if the dose received is less than 1.5 Gy (150 rads). However, if the dose is sufficiently high, a hematopoietic syndrome will appear after a few days. If the dose is even higher, the bone marrow suppression will be accompanied by a major gastrointestinal syndrome which is characterized by diarrhea and melena. If the subject survives these acute and subacute effects, he may subsequently develop radiation esophagitis or enteritis. In addition, the risk of gastrointestinal cancer and leukemia appears to be increased.

In the present paper, we will discuss only the changes of gastrointestinal motility observed during the prodromal syndrome.

1. Nausea and vomiting. These are the most prominent symptoms observed immediately after exposure to ionizing radiation. It is a complex process coordinated by the central nervous system and involving the respiratory and gastrointestinal systems. Vomiting is usually preceded and followed by hypersalivation. It can be produced by very low doses of total ^{60}Co teletherapy and even lower doses of neutron irradiation. In the dog, the ED_{50} for vomiting varies between 1.7 Gy[3] (170 rads) and 5.4 Gy[4] (540 rads). It is observed in 90 to 100% of the dogs exposed to 8 Gy (800 rads)[5].

The mechanism by which irradiation produces vomiting is unknown. It may be induced by irradiation of various parts of the body, although the effect is decreased if the upper abdomen is shielded[6,7]. Nonetheless, radiation may still elicit vomiting after complete excision of the stomach, small intestine and colon[8]. Radiation-induced vomiting is prevented by the surgical resection or thermal coagulation of an area of the medulla located immediately under the floor of the fourth ventricle, the chemoreceptor trigger zone (CTZ)[4,9]. In contrast, shielding of the CTZ does not prevent vomiting[10]. Based on these

observations, it is possible that ionizing radiations stimulate the CTZ indirectly, either through the activation of the peripheral end of afferent nerves or through the release of one or several humoral factors. By analogy with vomiting produced by apomorphine and certain cardiac glycosides, the CTZ would then send impulses to the vomiting center (VC), which is located in the lateral reticular formation. The VC would then initiate the complex motor events characterizing retching and vomiting, i.e. coordinated movements of the respiratory muscles accompanied by an inhibition respiration.

In the dog, vomiting caused by ionizing radiations can be effectively prevented by domperidone[5]. This protective effect is similar to that afforded by resection of the CTZ[4] and is compatible with a direct effect of the drug on that area of the brain. Indeed, domperidone had been shown to bind with cerebral dopaminergic receptors. However, since it does not cross the blood-brain barrier, it reaches only the areas of the brain that are outside this barrier[11]. Since the CTZ is known to have a "leaky" blood-brain barrier, it can be reached by domperidone. Since domperidone does not easily reach the striatum[11], it is devoid of extrapyramidal side effects such as the ones observed after metoclopramide. In addition, contrary to neuroleptic agents, it does not modify the state of vigilance. Thus, domperidone could be used to prevent radiation induced vomiting with no predictable side effects. Several studies performed in man have suggested that domperidone was indeed effective in decreasing nausea and vomiting encountered during radiotherapy[12]. Unfortunately, no double blind design was used and this shortcoming may be very important in a situation where psychological factors are known to be very important. Thus, it would be very important that additional studies be performed in man using double blind administration of domperidone and other antinausea agents and/or placebo.

2. Gastrointestinal motility. The mechanical and electrical activity of the stomach following exposure to radiation is largely unknown. In contrast, the effect of irradiation on small intestinal motility has been extensively studied in rodents[2]. An increase in tone and motility was observed immediately after exposure to doses as low as 100 rads. This effect appears to be related to the direct effect of radiation as it was prevented by shielding of the intestinal loop[2]. Vomiting produced by apomorphine or other stimuli is

preceded by slowing and/or suppression of electrical activity[13] and relaxation of the stomach, the latter being abolished by vagotomy[14,15]. Apomorphine induced vomiting is also preceded by initial suppression of the electrical activity of the duodenum followed by bursts of antiperistaltic activity[16,17]. As a result, intragastric pressure is decreased and intraduodenal pressure is increased[18]. The above mentioned changes in gastrointestinal activity following apomorphine are believed to be induced by central mechanisms involving the dorsal nucleus of the vagus, which are in the immediate vicinity of the CTZ and VC. Therefore, it is possible that stimulation of afferent nerves or the release of humoral factors that cause radiation-induced vomiting are also responsible for the activation of the vagal nuclei or other central nervous system centers. In turn, these centers would modify gastrointestinal motility either through the stimulation of the inhibitory fibers of the vagus nerve[14] or through the release of humoral agents.

Gastric emptying is suppressed within 20 min of exposure to ionizing radiation and remains low for at least 3 hours. This phenomenon is observed both in species that do not vomit such as rodents,[2,19,20], and in those that do vomit such as dogs[5] and monkeys[21]. In addition, intestinal propulsion is increased immediately after irradiation but is depressed during the subsequent 3 days[2]. The mechanisms of these effects is unclear. The radiation-induced suppression of gastric emptying is not prevented by pretreatment with domperidone. In contrast, this medication suppresses the delay of gastric emptying induced by apomorphine[23] or dopamine[24]. Therefore, the dopaminergic receptors of the CTZ or of the stomach do not appear to be involved in the radiation-induced delay of gastric emptying. In addition, since vomiting can be suppressed by domperidone without improvement of the radiation-induced delay of gastric emptying, it appears that the two symptoms are independent of each other.

3. Gastric secretion. In monkeys, whole body irradiation with gamma photons (8 Gy) abolishes basal and stimulated acid output from 40 min to two hours after exposure[21]. However, basal and stimulated acid output has returned to normal 2 days after irradiation[21]. In contrast, acid output is suppressed for 3 to 10 days in pigs receiving 1.1 Gy[25].

II. EFFECTS OF SURGICAL TRAUMA

1. Experimental Models

The effects of trauma and surgery has been extensively studied in rats, dogs and men[26-32]. A delay of gastric emptying was demonstrated in all 3 species. The abnormality causing this delay in emptying was demonstrated in all 3 species. The abnormality causing this delay in emptying is unclear but appears to be related to an inhibition of mechanical and electrical activity. In addition, acid output is enhanced in dogs following surgical trauma[27], which may explain the increased frequency of peptic ulcer and gastrointestinal bleeding observed after trauma[33]. However, it is possible that changes in blood flow also play a role in the production of gastrointestinal bleeding after surgical trauma[34].

2. Pathophysiology

a. Adrenalectomy. Circulating catecholamines are elevated after surgery[30]. However, adrenalectomy does not suppress the ileus observed after surgery, although it virtually abolishes plasma levels of catecholamines[30]. From these observations in the rat, it can be concluded that circulating catecholamines do not play an important role in the pathogenesis of postoperative ileus.

b. Chemical sympathectomy. Intravenous administration of 6-OH-dopamine produces complete sympathetic denervation of all the organs located outside of the blood brain barrier. It also completely prevents the appearance of postoperative ileus[28]. Thus, in contrast to the circulating catecholamines, the norepinephrine released at the sympathetic nerve terminals is necessary for the occurrence of postoperative gastrointestinal paralysis.

c. Sympathetic neuronal activity. Norepinephrine turnover reflects the sympathetic activity in a given organ. The rate of synthesis of norepinephrine can be measured following injection of its labelled precursor, ^{14}C-tyrosine.

Similarly, the rate of catabolism of norepinephrine can be measured by blocking the synthesis of norepinephrine with -methyl-para-tyrosine. By using both methods in rats in whom gastric emptying was suppressed following surgery, we demonstrated that sympathetic nerves were hyperactive at that time[28,29].

3. Treatment

In dogs postoperative ileus was prevented with bretylium[26], a drug that blocks the release of norepinephrine by nerve terminals. In man, the combination of - and -adrenergic blocking drugs was also effective in preventing or suppressing postoperative ileus[31].

SUMMARY

Exposure to ionizing radiation is immediately followed by important modifications of gastrointestinal function. The first symptom to occur is probably vomiting, which is accompanied by hypersalivation, coordinated activity of the respiratory muscles and inhibition of respiration. This vomiting is believed to be mediated by nervous centers located in the medulla, and can be suppressed by pretreatment with dopamine antagonists. Before and after vomiting, the functions of the gastrointestinal tract are markedly disturbed. Gastric motility is inhibited and intestinal propulsion is reversed, resulting in delayed gastric emptying. In addition, gastric acid secretion is suppressed. The exact duration of these symptoms is unknown but animals do not vomit after 3 hours (monkeys) or 6 hours (dogs) and gastric emptying and secretion is normal 2 days after irradiation with 8 Gy (800 rads).

Surgical trauma is also followed by profound changes in gastrointestinal functions. Initially, the stomach and intestines are completely paralyzed. Depending on the intensity of the trauma, this paralysis lasts between a few hours to several days. Small intestinal motility usually recovers first, while the stomach and colon remain inhibited for a longer time. In addition, acid output is increased which may be responsible in part for the high incidence of bleeding peptic ulcer in surgical and trauma patients.

The effect of combined trauma and irradiation on gastrointestinal function is unknown. It is expected, however, that the gastrointestinal tract will be paralyzed. In contrast, the effect on acid output is unpredictable, as trauma and irradiation have opposite effects.

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DISCUSSION PERIOD WITH DR. DUBOIS

DR. KAPLAN: In the first study you gave the dumperidone pre-radiation. Have you done any studies giving it afterwards to see whether the effect is the same or different?

DR. DUBOIS: No, we have not. But Dr. Court in France, has tried it. He found that the drug was less effective than when given before exposure. Apparently you need to give domperidone before exposure to afford maximum protection.

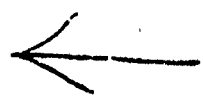
DR. HAFFORD: Is the radiation effect or the gastric ileus a dose related effect and, if so, is there a linear relationship? Have you studied the effect of lower doses?

DR. DUBOIS: No, so far we have not

DR. HIRSCH: Do you have any thoughts about acid production in the stomach?

DR. DUBOIS: The question has been addressed in recent studies performed in monkeys by Dr. Danquechin Dorval. We found in monkeys a suppression of gastric emptying like in the dogs. In addition we found that acid levels were suppressed.

Interestingly enough, there was a delay in the suppression of acid. That is, whereas emptying was suppressed, by the time the monkeys were back from the exposure room, it took an additional 20 to 30 minutes for acid suppression to be observed. I would like to comment about some additional results Drs. Dorval, Mueller and I have recently obtained in monkeys. Plasma beta-endorphin levels were found to have increased tenfold. Injections of endorphins at doses similar to plasma levels do not induce vomiting. These observations should be viewed cautiously as plasma levels may reflect release of endorphins very close to specific receptors, i.e. brain. Therefore, the intravenous injection of endorphins may not produce high local concentrations at the receptor site. To further evaluate this one should inject endorphin into cerebral areas sensitive to endogenous opiates such as the chemoreceptor zones or the vomiting center.



AD-P003 862

CARE OF THERMALLY INJURED VICTIMS OF A THERMONUCLEAR EXPLOSION

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INTRODUCTION

Nuclear war or accidental non-hostile nuclear explosion would obviously rank among the greatest disasters to affect mankind. In addition to massive numbers of casualties, depending on the circumstances, our ability to care for these casualties might also be affected. Despite the predictions of doomsday soothsayers, nothing is accomplished by burying one's head in the sand. Therefore, we must give consideration to how victims of a thermo-nuclear explosion would be cared for.

The burn patient, no matter what the etiologic agent of his injury, requires massive support in both personnel and supplies. Nursing and paramedical staffing of a burn center is frequently at a higher ratio than even an intensive care unit. Daily pharmaceutical and dressing supplies are frequently ordered by the case, even for an individual patient. There are currently under 2000 designated burn-care beds and approximately 175 burn centers in the United States. On any given day, approximately one-half to three-fourths of these beds are occupied; therefore, one would expect only 500 to 1000 readily available burn beds in this country. Obviously,other beds can obviously be used for the treatment of burn victims, but the treatment of burn patients outside of an organized burn-care program requires an even greater than usual personnel ratio.

Estimation of exact numbers of military victims in a nuclear conflict is difficult, but one can safely assume that the numbers would be in the thousands rather than hundreds. A nuclear explosion within the continental United States, depending on its location, could produce a great number of victims and destroy some of the ability to care for those victims. In addition, normal supply channels, including production, distribution, and transportation, would be expected to be impaired. Based on the above assumption, it is almost

inevitable that triage based on military rather than civilian principles would have to be instituted, whether or not the injuries were due to warfare or accidental nuclear detonation.

The severity of a burn injury is related primarily to the extent and depth of the burn wound. Other factors, such as age and the prior health of the patient, smoke inhalation, and associated injuries, may also affect the outcome of a burn injury.

The extent of a burn wound may be rapidly estimated or precisely calculated. Rapid estimation of the area of the burn wound is provided by "The Rule of Nine's." This technique divides the body surface into areas of approximately 9%. Each upper extremity (the hand, forearm, and upper arm) comprises 9%; the head and neck, 9%. Each leg comprises 18%, and this may be divided into the front and back surfaces or above and below the knee, 9% each. The anterior chest and abdomen comprise approximately 18% or 2 times 9%. Totaling these areas gives 99%; the additional 1% is primarily assigned to the groin. It must be emphasized that the rule of nine's applies only to adults, and it becomes more and more inaccurate as the age of the victim decreases below 15. With the use of the rule of nine's, however, a rapid estimation of the extent of a burn wound may be made. In patients with massive burn injuries, it may be more useful to use the rule of nine's on the unburned area and then subtract that percentage from 100%.

For more precise calculation of the percentage of burn wound surface area, the chart developed by Lund and Browder is utilized. This chart divides the body into multiple areas of varying small percentages. After the patient has been initially cleaned and debrided, the wounds are very carefully sketched out, and then an estimate is made of the percentage of the given area involved. For the arms, rather than taking 9% as the total estimate of the arm, it is broken down in the adult to 4% for the upper arm, 3% for the forearm and 2½% for the hands. Breaking it into the smaller areas allows a more precise estimation of the body surface area involved.

Once the extent of the burn wound has been calculated, it is important to know the depth of the burn wound. Burn wounds are generally classified in

three depths, namely, first, second, and third degree. Historically, as many as six depth classifications have been used, and today many people use four degrees of depth. Occasionally, the burn wound is defined only in terms of partial or full-thickness injury.

In the first-degree burn wound (sometimes referred to as superficial), partial thickness is the mildest form of burn injury. Only the most superficial layers of the skin (epidermis) are injured. The first-degree wound is red and mildly painful. It requires treatment only for alleviation of pain, and rarely has any medical significance. In a severely injured burn patient, first-degree wounds are generally ignored in calculating fluid resuscitation requirements and survival statistics.

Second-degree burn wounds compromise a very wide range of injury, from a relatively superficial burn, which will heal in a few days, through an extremely deep second-degree burn, which will require a skin graft for optimal healing. The skin is blistered or may be weeping profusely if the blister has been broken. These wounds are generally painful, are bright red in appearance, and blanch easily when pressed with a finger. The superficial second-degree wounds will heal within a few days to a week, and generally develop minimal scarring. There may be temporary changes in pigmentation or skin color, particularly in a dark-skinned individual, but normal color and skin texture return within a few weeks to occasionally a few month's time.

Moderate-depth second-degree wounds are generally quite painful, blistered, and red to pink in appearance. They are sensitive to pin prick. These wounds should receive medical attention, and generally take 10 days to 3 weeks to heal. Should the moderate-depth second-degree wound become infected, the wound may progress to a full-thickness or third-degree injury.

The deep second-degree or deep partial-thickness injury is the deepest burn wound from which spontaneous healing is possible. The deep second-degree wound is mottled pink or white, but may not blanch on pressure. The skin still has a relatively normal consistency on palpation. These wounds are frequently

anaesthetic to pin prick and generally are not painful; however, there may be pain associated with this injury. Small areas of deep second degree may be allowed to heal spontaneously; however, this healing will take 3 to 6 weeks or more, and nearly always produces significant scarring. The deep second-degree burn is frequently treated by early excision and split thickness grafting of the burn wound.

Third-degree injury, or full thickness, is complete destruction of the skin. This wound is charred, brown, or pearly white in appearance and will not change color on pressure. The skin has the feel of leather, or parchment, with very little or none of the stretch of unburned skin. These wounds are anesthetic, as the nerve endings have been destroyed. Third-degree wound always require medical attention, and if larger in size than a quarter, they will nearly always require skin grafting. Third-degree wounds always leave residual scarring, even if a "perfect" graft procedure is performed.

The term fourth-degree burn may occasionally be seen. Those who use this term imply that the burn involves muscle, bone, or other deeper structures. This term, however, is not widely used or universally accepted.

Without additional factors, a patient with a burn of 15-20% or less of body surface can usually be resuscitated with only oral fluids, and could be treated on a outpatient basis if absolutely necessary, even though this might not be optimal under the usual circumstances. Victims at the other end of the spectrum with third-degree burns in excess of 40% of the body surface or total burns in excess of 60% of the body surface are unlikely to survive without massive and optimal medical and surgical support. Unfortunately, I have to recommend that they not be treated at all in a mass disaster situation. Likewise, those victims with burns and/or injuries and severe smoke inhalation injury will require massive support, and must be triaged to a non-treatment or comfort-treatment group. It is those patients with burns between 15% and 60% for whom feasible care allows a reasonable chance of survival.

CARE OF THE VICTIM OF A COMBINED NUCLEAR AND THERMAL INJURY

Acute burn care begins with an assessment of the burn victim for associated injuries. No matter how extensive, an acute burn wound is not immediately life-threatening. Smoke inhalation and intoxication, or airway obstruction due to trauma to the face or neck, or massive exsanguination, whether external or internal, will result in the rapid demise of a burn patient. In a mass disaster situation, however, it is unlikely that these patients would survive until they can be reached by paramedical or medical personnel. But if they do survive, then immediate, on-the-scene care is absolutely mandatory, no matter how minor the burn injury is. In addition to thermal and physical injuries, victims of a nuclear explosion would also sustain radiation injuries of varying degrees. Victims close enough to a conventional weapon or within the sphere of an enhanced radiation weapon would manifest symptoms of radiation exposure that depend on the actual received radiation dose. Victims receiving rapidly lethal radiation doses would probably exhibit radiation sickness by the time they are reached, and would be triaged into expectant groups. Those patients receiving eventually (but not immediately) lethal exposures would not appear different from non-radiated burn victims and would receive immediate care in an identical fashion.

Initial medical care of radiated burn victims would be similar to that of non-radiated victims. Clothing and debris on the victim might be contaminated with products of fission, and need to be decontaminated in a fashion identical to that in a non-burned but irradiated or contaminated victim. Removal of clothing and showering or bathing the patient will remove all significant external radiation sources.

The initial care of a burn victim beyond assessment and correction of immediately life-threatening associated injuries lies primarily in fluid resuscitation. Many formulae for fluid resuscitation have been proposed. While there have been considerable research and clinical presentations as to the necessity or value of colloids in initial resuscitation, the current consensus is that an adult patient may be adequately resuscitated without the use of colloid-containing solutions such as plasma or plasmanate. Both

slightly hypotonic and hypertonic formulae are currently in use. Resuscitation with Ringer's lactate in quantities of 3-4 ml/percent of body surface burns/kg of body weight provides excellent restoration of circulating volume that has been depleted through third space losses. The advantage of this resuscitative formula is that Ringer's lactate is a standard readily available fluid, and if the urine output is titrated to 40-60 ml/hr in the adult, significant blood chemistry abnormalities are unlikely to occur. The disadvantages of the Ringer's lactate resuscitation are the massive volumes of fluid required and the swelling that may occur, necessitating escharotomies of the extremities to prevent acute compartment syndrome. In addition, the swelling may result in massive facial or neck edema, necessitating intubation or tracheostomy for airway management.

Hypertonic resuscitation using HLS (hypertonic lactated saline) has been advocated to provide the necessary sodium without excess water. This solution is made by adding 100 mEq of sodium lactate to 1 liter of normal sodium chloride. The advantage of hypertonic resuscitation is that swelling is minimized, thereby minimizing or eliminating the need for escharotomies and decreasing the incidence of ileus and respiratory distress due to pulmonary edema. The disadvantage to hypertonic resuscitation lies in the need to carefully monitor electrolytes, which may be difficult in a mass casualty situation. The estimated volumes for HLS are calculated on the basis of 0.55 mEq of sodium/kg of body weight/percent burn, but the urine must be carefully titrated to 30-50 ml/hr.

From the above discussion, one can infer that either Ringer's lactate or hypertonic lactated saline is a suitable resuscitation fluid if the normal hospital system is intact. On site in the field, or if a nuclear explosion occurs within the continental United States, Ringer's lactate would be preferable, due to its ready availability as a standard fluid and the greater leeway for imprecise administration.

Indwelling intravenous lines provide a colonization site for bacteria during the transient bacteremia produced by manipulation of the burn wound. Therefore, the lines should be placed in conditions as close to sterile as possible. In addition, these lines should be removed as early as possible, and

the patient placed on oral maintenance as early as a functioning G.I. tract will allow. Peripheral intravenous lines are frequently difficult to start in burn victims, and central vein catherization may be necessary. If a central line is begun under less than sterile conditions, it should be replaced as soon as practical upon arrival at a hospital setting.

Foley catheterization of the bladder should be performed on patients with burns greater than 20%, but if supply is a problem, measurement of urine output may be done by encouraging the patient to void approximately every 4 hours. Those patients with burns greater than 40% body surface or those with burns of the perineum must have an indwelling catheter inserted as early as possible, as late catheterization may be difficult if not impossible.

Nasogastric intubation will be required on those patients to be air-evacuated; otherwise, this may be left to the discretion of the treating physician.

CARE OF THE BURN WOUND

Care of the burn wound in the burned and radiated patient is no different from any other burn patient. As soon as possible or practical, the victim should be gently bathed to remove loose non-viable skin, remnants of burned clothing, and external debris, which may or may not be contaminated with radioactive products. Standard techniques of collection and disposal of contaminated materials should be utilized. Although residual radioactivity due to fission products and fallout may be present on the burn victim, once preliminary decontamination by washing has been carried out, the residual radiation will have minimal if any significant effect on medical personnel. So all standard procedures should be carried out as though the patient were a normal thermally injured burn patient.

At the scene of the casualty, no specific burn wound therapy is necessary. If facilities are available for cleansing the burn wound and applying a topical chemotherapeutic agent, silver sulfadiazine is currently the most useful anti-microbial agent. This agent can be smeared on the burn wounds using a sterile or clean gloved hand, and treated with either open or occulusive dressing.

These dressings should be changed daily or every other day, depending on the amount of exudation present and also on the availability of the topical agent and/or dressings. While the patient who has been exposed to lethal or sublethal radiation is more prone to succumb to infection than is a similar non-radiated patient, the initial wound care and appearance will be identical to those of the non-radiated burn victim.

Concomitant injury may be expected in the patient who has been burned and irradiated. Lacerations and fractures are likely due to the blast injury. Treatment of these associated injuries should follow standard military medical practice. Grossly contaminated lacerations should be gently cleaned, debrided, and dressed open. Fracture should be reduced, and held by either external splints or pins incorporated into traction or external fixation devices. Circular casts must be avoided because of the predictable swelling during resuscitation as well as the need to frequently examine burn wounds.

DEFINITIVE HOSPITAL CARE OF THE BURN VICTIM

Definitive acute hospital care of the burn victim is marked by a comparatively long course of very specialized, if not intensive, care. Daily wound care consists of meticulous cleansing of the burn wound, gentle debridement of non-viable tissue, frequent surgical intervention to debride or excise the burn wound, followed by skin grafting. As previously mentioned, this care is labor-intensive, and requires a very large store of supplies. Early surgical excision and grafting of the burn wounds provides a means for earlier burn wound closure and therefore decreased morbidity and mortality from a given injury. Unfortunately, this technique of early excision requires a high level of operating room support, and results in blood loss approximating one unit per percent of body surface excised. In selected patients with small, localized, deep burns, this technique is the procedure of choice in a mass disaster situation. However, its utilization would probably not be feasible for those patients with moderate-sized burns.

Infection is the most frequent and most significant complication that any burn patient faces. Victims of a thermonuclear explosion would be at particular risk for infection due to multiple factors. Embedded foreign

material, as might be driven into wounds by a blast effect, is well documented to increase the risk of infection. Delay in obtaining proper and adequate medical care may allow progression of an initially contaminated wound into an early infected wound, thereby necessitating therapeutic rather than prophylactic care. The mass influx of casualties would undoubtedly overload most hospitals or medical systems and aseptic technique would likely be compromised. The patient load for each medical/paramedical personnel would significantly increase the risk of hand-to-hand cross-contamination, and it is this source that is the most frequent form of burn wound contamination. Antibiotic availability for treatment of established infections might be compromised as well.

Radiated burn victims who do not sustain a rapidly lethal CNS syndrome may develop gastrointestinal or hematopoietic syndromes. In addition to the hemorrhage and fluid loss of the gastrointestinal syndrome, transient bacteremia due to impairment of mucosal barrier integrity may occur. Coupled with the propensity of the burn patient to develop life-threatening infections, the decrease in ability of the radiated patient to mount an appropriate cellular mediated response to infection may result in a significant increase in mortality and morbidity.

All other aspects of burn wound care in the burned radiated patient are similar to those in the burned non-radiated patient. Delay in wound healing is accelerated in the burned radiated victims, and slight breakdown and possible carcinogenesis of burn scars may occur, but the basic care is unchanged.

In summary, care of massive numbers of burned irradiated victims is a logistic rather than medical problem. Given adequate supplies and personnel, the care is not difficult, but it would need to be adjusted, depending on availability of care facilities. For nuclear as with all other types of burn etiologic agents, prevention is far preferable to treatment.

DISCUSSION PERIOD WITH DR. KAPLAN

DR. HIPSCH: I have a question for Dr. Carmichael. What medical preparedness, if anything, was organized, or what type of instructions were given by the British Command to the Medical Corp regarding possible massive numbers of burn casualties?

DR. CARMICHAEL: I think we would have, been hopelessly overwhelmed if we had received massive numbers of burn casualties as could be associated with the use of limited tactical nuclear weapons. As it was, over 14 percent of our casualties were burns due to missile attacks on ships. We had a problem of dealing with, on one occasion, 159 burn patients arriving in 3 hours. It was a logistical problem. There weren't any technical difficulties in treating the burns.

We would have, I think, been hopelessly overwhelmed if we had been in that situation. As it was, over 14 percent of our casualties were burns due to missile attacks on ships. Logistically we had a problem there of dealing with, on one occasion, 159 burn patients arriving in three hours. It was a logistical problem. There weren't any technical difficulties in treating the burns.



AD-P003 863

THE MANAGEMENT OF SOFT TISSUE INJURIES IN THE COMBINED INJURY PATIENT

ERWIN F. HIRSCH

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INTRODUCTION

The management of soft-tissue injuries has challenged trauma surgeons since the early times of the contemporary era. However, despite the advances in resuscitation, surgical techniques, antibiotics, etc., the simultaneous development of sophisticated weaponry of war, increased incidence of motor vehicular and industrial accidents, and increased incidence of crime have kept the issues of soft-tissue-injury management in the forefront of trauma surgery. Moreover, the possibility exists, however remote, that with the greater use of domestic and foreign nuclear energy, major industrial accidents could occur. Also the misuse of radioactive material by untrained hands may generate a combination of general trauma in association with total-body irradiation, which challenges trauma surgeons to develop strategies for the care of these patients.

The physiological response to a combined-injuries scenario in humans is by and large unknown since few cases have ever occurred. However, extensive animal experiments indicate that non-lethal radiation injuries convert to a lethal or near-lethal model when associated with conventional trauma or burns. The mechanism of death of these animals is characterized by uncontrolled sepsis and other classical manifestations of the acute radiation syndrome.

The purpose of this discussion is to address the specific procedures associated with the management of soft-tissue injuries and to incorporate thoughts applicable to the combined-injury scenario.

MANAGEMENT OF SOFT TISSUE INJURIES

Conventional management of significant soft-tissue injuries is characterized by the following:

Appropriate resuscitation and full evaluation of injuries in the Emergency Department
Early intraoperative repair of vital structures with thorough debridement and profuse irrigation of all areas
Discussion of the appropriate timing and methodology of wound closure.

The experience gained in the Second World, Korean, and Vietnam Wars, as well as in civilian trauma centers, clearly indicates that the time interval between injury and operative management is related to the likelihood of the development of septic wound complications and mortality.

A number of Eastern European investigators, looking into the issues of the combination of different types of trauma and radiation, established that for these experimental models, the optimal window during which surgical procedures can be successfully performed is different than for conventional trauma. Some of their experience indicates that the surgical correction of life-threatening and other injuries should be carried out as soon as possible following the combined injury (0-4 days) and that more elective "procedures" should be postponed until late in the convalescent period (2 to 3 months).

Established guidelines for the management of victims of skeletal trauma and total-body irradiation indicate the principles of resuscitation: Airway, breathing, and circulation should supercede all other clinical considerations, with the exception of a simultaneous attempt to determine the radiation dose the victim received by dosimetry, early blood count, or detailed recording of the signs and symptoms of the acute radiation syndrome.

Interoperative management of soft tissue injuries is characterized by the following:
Control of hemorrhage
Repair of vital structures
Conservative excision of the necrotic skin
Aggressive debridement of poorly profused subcutaneous tissue with removal of foreign bodies and clots
The exposure of muscle groups by incision of fascial plains
Aggressive debridement of all non-contractile muscle

Copious irrigation
Quantitative bacteriological samples of the wound

Unfortunately, the extent of debridement cannot always be determined at the time of initial therapy. This problem is more acute when the injuries are produced by missiles or crushing mechanisms. In those instances, it is appropriate that the patient be returned to the operating room within 48 hours from the initial surgery for wound inspection and further debridement if necessary.

WOUND CLOSURE

Primary closure of soft tissue injuries is never to be practiced in combat surgery, and it is of questionable validity when the mechanisms of injury are such that kinetic energy will result in swellin and/or edema with compromise of the circulation. Furthermore, in the event of loss of skin, attempts to approximate skin edges uniformly results in further skin necrosis.

The practice of delayed primary closure extensively developed during the Second World War is still the most common option for dealing with some of the large soft-tissue injuries. However, in order to approximate the skin edges without tension, 4 to 10 days may elapse between injury and the closure, which is a window of time that seems inappropriate in the patient with a combined injury.

Other alternatives of wound closure include the use of flaps, for which specialized training is required, or the use of biological dressings.

The closure of the traumatic wound emerges as the most challenging of all surgical therapeutic efforts in the combined-injury patient. This challenge is characterized by the relatively small period of time following injury when surgery can be safely performed, and the experimental data indicating the increased mortality in the absence of wound closure.

A clinical protocol that is not new to the non-military trauma surgeon could be the foundation for the care of the combined-injury patient. This protocol would (1) aggressively resuscitate the patient to minimize any

additional deleterious effects of hypovolemic shock in the combined-injury patient; (2) proceed with appropriate surgical procedures for specific injuries, including soft tissue injuries (3) return the patient to the operating room in 48 hours, obtain quantitative cultures, and if the wound is clinically clean, proceed to graft all defects with autologous skin; and (4) remove dressings at 96-120 hours, and if the wounds were appropriately debrided, skin grafts should have closed the wounds.

In the event that further debridement is necessary at 48 hours, the previous sequence of procedures could begin at 96 hours.

Death following thermal injury, if not associated with failure of resuscitation or inhalation, is associated with burn wound sepsis. The excision of a potential area from which sepsis may propagate and the early closure of the wound should serve as a basis for the development of experimental models in total-body radiation and burns.

SUMMARY

In summary, the management of soft tissue injuries should be characterized by (1) aggressive resuscitation, (2) dosimetry, (3) debridement of soft-tissue injury, and (4) return to the operating room at 48 hours for further debridement if necessary or closure of the wound with a split-thickness skin graft.



AD-P003 864

COMBINATION OF THERMAL BURNS AND WHOLE-BODY IRRADIATION

WRITTEN FOR JOHN F. BURKE BY ERWIN F. HIRSCH

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INTRODUCTION

Despite significant advances in medical technology (such as recognition of physiological derangements, use of topical bactericidal agents, and improved surgical techniques), the care of the thermally burned patient remains a constant challenge to the medical profession. Review of the mortality and morbidity from burn centers across the United States indicates that mortality is usually associated with failures of resuscitation, a significant inhalation injury accompanied by pulmonary insufficiency, or one of the different manifestations of sepsis in the burn patient.

Systemic sepsis in the burn patient that is not due to nosocomial infections or pulmonary injury is usually associated with a colonized or infected burn wound. Burn wound sepsis in some degree is present in most burn wounds beyond the 5th day regardless of topical therapy. However, burn wound sepsis manifests itself only if the bacterial count exceeds 10^5 organisms per gram of tissue. 100,000

Radiation injuries as a result of industry, accidents, or warfare have occurred during the past 40 years. The combination of thermal burns and whole-body irradiation (WBI) is a real concern in the event of war, terrorist attack, military accidents or a number of industrial mishaps related to the nuclear industry. The synergistic effects of burns and WBI have been demonstrated by several investigators. Standard burn models in animals with a mortality of 20%, when combined with sublethal doses of radiation, result in significant mortality usually associated with sepsis.

CONTROL OF BURN WOUND SEPSIS

Control of burn wound sepsis has traditionally been attempted by the use of topical bacteristatic agents developed in the past 30 years. Despite the increased use of such agents, control of burn wound sepsis has not been uniformally successful. Furthermore, these topical agents, some of which are toxic in nature, have not totally sterilized the burn wound.

Management of the burn wound can be either aggressive or conservative. Conservative management of the burn wound requires a period of 3 to 4 weeks before deep partial-thickness burns are healed or the eschar of deeper wounds has matured sufficiently so that separation occurs and a viable recipient surface for an autograph is available. The physiologic and metabolic consequences of this period of time between burn and wound closure have been well described, and are characterized by a constant catabolic state requiring significant metabolic support to prevent sepsis or multi-system failure.

Since 1974 the concept of early excision of the burn wound has regained scientific and medical acceptance as described by Burke and his associates.[1] During the past 8 years, a number of centers throughout the United States have adopted the early excision programs for the care of the burn patient. Simultaneously, other major burn centers have indicated that this type of aggressive management does not significantly alter the course of similar burned patients treated in a more conventional fashion. The indications at the present time, in those centers supporting the concept of primary excision of the burn wound, are full-thickness burns or deep partial-thickness burns that require more than 3 weeks to heal. The procedure requires immediate autologous skin grafting and the availability of skin from the patient or other materials to close the wound. Consequently, the availability of skin to close the wound determines the magnitude of the excision. The operative procedures require specialized equipment, specialized anesthetic, knowledge of unique surgical techniques, and significant operating room time. The results of such techniques have been analyzed by the retrospective review of patients as well as by experimental models. Hunt, Baxter, and others reviewed their

experience in split-thickness skin grafting of deep burns of the upper extremity, and concluded that this procedure accomplishes the preservation of tissue, prevention of wound infection, maintenance of function, and early wound closure.[2]

Heimbach and his associates reviewed their experience on early surgical excisions of the burn wound by comparing burn patients who sustained comparable injuries, they concluded that early excision of the burn wound significantly decreased burn wound colonization, burn wound sepsis, the number of days the patients remained on Chloramphenical, and length of time in hospital. However, they concluded that the patients who underwent early excision required significantly larger volumes of transfused blood.[4] The immune and metabolic response in the standardized burned guinea pig was addressed by Burke and his associates.[5] The results established that animals whose burn wounds were excised and then closed sustained much less weight loss, compared to those who were burned and the wound remained untouched. The animals that underwent excisions were free of sepsis. Those that were burned and cared for with topical silvadene developed wound sepsis at day 8. Those that were burned and received no treatment developed wound sepsis at day 5. The immune response of these animals was evaluated by comparing of the weight of the thymus and the relative thymus activity by establishing DNA synthesis. Thymus weights were significantly different in the burned, excised. closed animal when compared with the other models. Simultaneously DNA synthesis was significantly increased in those animals that underwent excision from the 8th through 12th day after burn.

The available literature on burns indicates that the rationale of primary excision of the burn wound is challenged by several investigators. The belief of these investigators is that deep partial-thickness burn wounds will heal adequately without functional defects if treated with appropriate topical antibacterial therapy. With proper topical care, full-thickness burns can be allowed to mature and then be grafted when necessary, without changes in mortality or morbidity.

It is not the point of this presentation to challenge or argue the care of the non-irradiated burn victims. If one accepts the concept that sublethal radiation exposure when associated with a sublethal burn has an increased mortality in animal systems, then early excision of potentially septic tissue and closure of the wound should improve the outcome. The logistics and validity of such a program for treatment of combined injuries, the distribution of resources, and the feasibility of practicing this type of clinical activity in the presence of multiple casualties requires further research, development, and establishment of appropriate policies.

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DISCUSSION PERIOD WITH DR. BURKE

Editors' note: Dr. Burke presented material concerning the problem of burn wound sepsis, but was unable to provide a manuscript for this publication. Therefore, Dr. Hirsch prepared the preceding summary of this topic.

DR. CAMP: Dr. Burke, have you scaled your dermal equivalent up to industrial production yet so that it can be mass-produced?

DR. BURKE: The Marion Laboratories have scaled it up to industrial production, but have been prevented from dispensing it by the FDA. I hope that within the very near future we can begin to send it out to organized centers so we can get experience in addition to ours in Boston.

DR. CAMP: Do you know anyone who is working on maggot enzyme systems?

DR. BURKE: No I don't, nor had I thought of maggot enzyme systems before our friend from Holland showed us.

DR. MESSERSCHMIDT: I know from our experiments with irradiated animals that the open wound is very dangerous. If you close the wound, our animals will survive. I know that war wounds cannot be closed. Therefore, we are always looking at any method to close wounds in an irradiated individual.

DR. BURKE: I think we have all learned from experience, in military disasters and otherwise, that you get into sepsis troubles by closing wounds that ought not to be closed. The important point here is that when a surgeon talks about closing a wound, he is talking about closing it in a definitive way, both anatomically and physiologically. Perhaps that is more complicated than is needed to do immediately after an injury, because we do not have to solve the anatomic problem immediately in the wound closure area, except possibly in the aortic area. In skin wounds we do not have to solve the anatomical problem immediately. However, you do have to solve the physiologic

problems. If you do not solve the physiologic problems, then we are off to the septic races. I want to be sure that you understand that there are a lot of approaches, with artificial skin being one.

DP. HIRSCH: This will not work unless all of the necrotic skin is excised. Yes, it is true that war wounds or high-velocity wounds cannot be closed. However, in addition to learning how to cover the wound, we are going to have to better learn how much one has to excise. Unless the excision of the devitalized tissue is complete, neither that coverage nor any other coverage is going to work.

We may need to change our terminology, redefine our definitions, and reach within the first 24-48 hours a viable physiological surface to which some type of wound closure can be applied. That surface might be artificial skin or autologous graft. But we must not go back to the traditional wound closure, which some people think is just putting two edges of skin together.

DR. BAINES: With the exception of a full-thickness burn, what is your posture toward treatment of the site of injury until you get to some type of expert help? In particular, I refer to antibiotics, a temporary cover, and keeping the wound moist versus dry.

DR. BURKE: Some kind of physiologic cover has to be put on. And unless you can get rid of all of the dead tissue, as Dr. Hirsch has said, you have got to do something to prevent the bacterial growth in that area. Although I have no experience with antibiotics and artificial skin, for instance, I think that may be one approach. There are plenty of bacteria in the world, however, and there is no antibiotic that I know of that will kill them all off. It may be better than nothing, but it is not going to be a definitive solution. Regarding wound closure, we must re-establish normal physiology rather than anatomic integrity immediately. You have to do something to mechanically close the wound plus make any necessary biologic additions.

AD-P003 865

PREVENTION OF ISCHEMIA-INDUCED ACUTE RENAL FAILURE

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INTRODUCTION

Mannitol, furosemide, prostaglandin E_2, and verapamil have been demonstrated to be protective against ischemic acute renal failure ~~(Table 1)~~. Although the functional improvements in such renal parameters as glomerular filtration rate and tubular reabsorption of sodium are clearly demonstrable after therapy, the cellular mechanism(s) that underlie the decreased renal function are not well understood. These pharmacological agents exert multiple physiological effects, and it has been difficult to attribute their protective effect(s) to a common mechanism.

However, new insights into the protective role of each of these drugs have been derived from our sequential studies of mitochondrial function during and following ischemia in both the intrarenal norepinephrine model (NE; 0.75 mg/kg/min) and the renal artery clamp model (45-50 min) of ischemic acute renal failure (ARF) in dog and rat, respectively. In the presence of an adequate supply of oxygen, mitochondria serve a primary role in energy metabolism in normal cells. Oxygen deprivation during ischemia prevents high energy phosphate (ATP) generation, i.e., oxidative phosphorylation, by mitochondria. Studies from our laboratory have examined mitochondrial function during ischemia and from 0 to 24 hr after reflow. We chose 40-50 min of ischemia since shorter periods do not result in ARF and longer periods usually result in irreversible ARF. Therefore, this approach appeared to closely mimic reversible ARF in man. The experiments were designed to demonstrate protection or attenuation of the renal ischemic damage.

Our focus on mitochondrial function was based on the supposition that ischemia may prevent mitochondria from forming sufficient ATP to permit full restoration of cellular functions. For example, with little or no ATP, cell

membrane pumps, which maintain normal ionic homeostasis, cease to function. As a result, cells gain Na^+, Cl^-, and Ca^{++}; lose K^+ and $PO_4^=$; and swell. Swollen cells, especially in vascular tissue, may contribute to the reduced renal blood flow of ischemic ARF. Swollen tubular epithelial cells may form blebs and colliculi, which contribute to the casts and tubular obstruction that characterize ischemic ARF.

Finally, the oxygen-dependent loss of ATP synthesis and the concomitant increase of intracellular Ca^{++} may activate calmodulin and phospholipase activity. This latter event is expected to contribute to the process of cell necrosis. For these reasons, in addition to mitochondrial respiration, we also examined mitochondrial Ca^{++} content, uptake, and release in normal and ischemic kidney tissue.

MATERIALS AND METHODS

Mongrel dogs and Sprague-Dawley rats were used in these studies of ischemic ARF. In the dog, unilateral renal artery infusion of norepinephrine (NE; 0.75 mg/kg/min) for 40 min resulted in reversible ischemic ARF. In the rat, bilateral renal artery and vein clamping for 45-50 min was used to create the reversible ischemic ARF model. In the rat model, both kidneys were insulted in order to provide enough renal tissue to perform the mitochondrial studies. In the dog, the cortex of the ischemic and contralateral kidney was separated from the medulla. After scissor mincing in the cold and subsequent centrifugation, a very pure preparation of mitochondria was obtained, enriched 5-6 times over the original homogenate. In the rat, the papilla was separated from the kidney, and the remaining kidney tissue including cortex and outer medulla was prepared as described above. Sham-operated animals served as controls.

Mitochondrial function was assessed by measuring State 3 respiration (ADP stimulated, oxidative phosphorylation), State 4 respiration (succinate stimulated), and uncoupled (FCCP) respiration. Oxygen electrode methodology was used to assess rates of O_2 consumption.

Ca^{++} content of mitochondria and renal cortical tissue was determined by atomic absorption spectroscopy. Ca^{++} uptake and release rates by normal and ischemic mitochondria were determined by Ca^{++} electrode methods.

Longitudinal studies were statistically compared by regression analysis; in addition, paired and unpaired Student's "t" tests were used when appropriate. Values reported are mean $\pm$ 1 SE, and significant differences occur when $p < 0.05$.

RESULTS

In Table 1 are shown the GFR and RBF recovery patterns after various treatments that prevent this NE model of ischemic ARF. Although in many studies no data at 24 hr are presently available, it is generally assumed that the recovery, seen at 3 hr, will be sustained in these models, as has been demonstrated for mannitol and verapamil.

TABLE 1. EFFECT OF VARIOUS DRUGS ON THE PREVENTION OF NE-INDUCED ISCHEMIC ARF

	GFR (ml/min)				RBF (ml/min)				
	Pre NE Control	Post NE (hr) 1	3	24	Pre NE Control	Post NE (hr) 1	3	24	Ref
None	22	2	4	3	221	87	138	148	(1)
Furosemide* (pretreatment)	50	-	28	-	296	-	374	-	(2)
Furosemide (posttreatment)	58	-	15	-	322	-	281	-	(2)
Mannitol* (systemic)	26	1	8	18	220	70	174	191	(1)
Mannitol* (intrarenal)	34	-	9	24	345	207	178	205	(3)
Verapamil* (pretreatment)	29	16	-	31	270	200	-	181	(4)
Verapamil* (posttreatment)	24	14	-	18	225	210	220	-	(4)
Prostagladin E_2*	38	-	20	-	166	-	101	-	(5)
Bradykinin*	45	-	25	-	290	-	104	-	(6)
Secretin	35	-	4	-	282	-	144	-	(6)
Acetylcholine	44	-	6	-	255	-	262	-	(2)

*Protection to GFR demonstrated at 3 or 24 hr post ARF

We have also examined mitochondrial respiratory function and Ca^{++} handling after ischemic ARF. These data are presented in Table 2. Clearly, both mannitol and verapamil exert substantial protection to renal cortical mitochondrial function at 24 hr post ischemia, a time when functional improvement is also seen. The results, however, did not distinguish whether (1) the poor mitochondrial function and Ca^{++} overload seen at 24 hr were simply the result of cell death, or (2) the Ca^{++} overload actually caused the mitochondrial respiratory dysfunction and thus cell death. Our next series of studies sought to examine these two questions.

TABLE 2. MITOCHONDRIAL RESPIRATION AND Ca^{++} HANDLING AFTER NE-INDUCED ISCHEMIC ARF

	MITOCHONDRIAL RESPIRATION (natoms O_2/mg/min)			MITOCHONDRIAL CALCIUM ($nmoles\ Ca^{++}$/mg/min)		(nmoles/mg)
	S4	S3	FCCP	Uptake	Release	Content
Control	22	115	210	360	0.47	44
NE 24 hr	6	16	30	30	23.00	101
Mannitol 24 hr	15	72	107	198	0.70	55
Verapamil 24 hr (pretreatment)	19	115	107	305	0.60	43
Verapamil 24 hr (posttreatment)	18	100	171	325	3.00	52

To answer the question of whether Ca^{++} overload precedes mitochondrial dysfunction or vice versa, we conducted a series of studies in rats. Bilateral renal artery clamping was undertaken for 45 min. This ischemic insult resulted in a progressive rise in serum creatinine and blood urea nitrogen, which peaked at 24 hr. There was a clear ($r = 0.81$) inverse correlation between the rise in serum creatinine (at 1, 4 and 24 hr) and the fall in mitochondrial respiration; oxidative phosphorylation (State 3) was most severely inhibited.

A strong correlation ($r = 0.89$) was also found between the decrease in respiration and the rise in mitochondrial Ca^{++}. Thus, there was a parallelism between the decreased mitochondrial respiration and mitochondrial Ca^{++} accumulation over the entire 24 hr post-ischemic period.

The relative importance of mitochondrial Ca^{++} and mitochondrial respiration therefore becomes important. If, indeed, this mitochondrial Ca^{++} accumulation, which appears to occur during ischemia, prevents adequate mitochondrial respiration, then it should be possible in the absence of ischemia to load mitochondria and to observe a reduction in mitochondrial respiration. We have performed these studies in a model of chronic (8 days) hypercalcemia in normal rats. This procedure results in a two- to threefold increase in mitochondrial Ca^{++} and a significant although not lethal decrease in oxidative phosphorylation. It seems possible, therefore, that the overload of mitochondrial Ca^{++} following ischemia is the cause of the reduced mitochondrial respiration. If this is so, then prevention of Ca^{++} overload by calcium membrane blockers (i.e., verapamil) before or after ischemia may prove to exert a protective effect and enhance recovery from ischemic renal injury.

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DISCUSSION PERIOD WITH DR. SCHRIER

DR. SCHRIER: Verapamil was given afterwards. We have given furosemide and mannicol afterwards also.

It is clear that you have to intervene early. In all of the clinical studies, it was found that if one waits until the creatinine rises to six or eight, one can give almost anything they want and cannot attenuate the injury.

We have 40 patients that were given furosemide and 18 of them converted to non-oliguric renal failure and had a mortality of 20 percent versus 50 percent in the patients with oliguric renal failure. Morbidity was much lower in the non-oliguric patients. The ones that converted had creatinines around three. The ones that did not convert had creatinines around 5.5.

So it appears that one has to make a diagnosis early using urinary indices and then intervene. We may not have the right combination yet. The right combination may be both mannitol and Verapamil. It could also be Dopamine and mannitol, or chlorpromazine, or magnesium ATP. There are a number of potential clinical studies that I am sure will be initiated within the next few years.

DR. CATRAVAS: You mentioned that the levels of ATP are very much reduced. Since there are three different sites of ATP formation in the electron transport chain, I was wondering whether you have attempted to determine which of these sites is affected.

DR. SCHRIER: We have not done that yet, but it is on the drawing board.

IMMUNOLOGICAL AND INFLAMMATORY RESPONSES

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ROLE OF RETICULOENDOTHELIAL AND ENDOTHELIAL CELLS IN RESPONSE TO TRAUMA AND SHOCK

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INTRODUCTION

Ever since the classic studies of Blalock in the 1930's,[1] it has been recognized that the clinical picture of circulatory shock is "dependent upon an inadequate blood supply to the tissues of the body," and that failure of the microcirculation has been said, by many, to be the pivotal event in the shock syndrome (see references 2-6, for recent reviews). But, if we do indeed recognize the latter as a sinequanon, then why can't we adequately treat and manage all patients effectively? Moreover, why can't we prevent the sequelae of agonal events (Table 1) that take place secondarily to surgery? In addition, why, in particular, are the lungs, liver, kidneys, and brain so vulnerable to permanent damage in low-flow states subsequent to trauma and surgery?

Although there is at present no agreement among "shock experts" as to the etiological or sustaining mechanisms of low-flow state syndromes, evidence has accumulated to suggest that circulatory shock induced by such diverse factors as trauma, hypovolemia, cardiac tamponade, and septicemia, among others, often results in renal failure, liver failure, and a condition known as "shock lung." The compromise of such diverse organ systems has led to the concept that shock is a condition of multiple organ failure.[3,6] Since the intimal surfaces of the vascular system in the form of endothelial cells (EC) and reticuloendothelial system (RES) cells are vital to preserve (and protect) the integrity of organs and tissues, it must be entertained that these cell types could be pivotal in the etiology and progression of shock syndromes, as has been suggested recently.[3,6-11]

This paper will focus on the importance and interaction of EC and RES cell function with microcirculatory integrity and function in shock and trauma.

RETICULOENDOTHELIAL SYSTEM FUNCTION IN SHOCK AND TRAUMA

Since the characterization, morphology, ultrastructure, immunochemistry, pathophysiology, and biochemistry of the RES are important conceptually and are beyond the space limitations of this review, the uninformed reader should consult several reviews and monographs.[7,8,12-14] Of all the physiologic functions (e.g., host defense, macrophagia, destruction of microorganisms, removal of circulating tumor cells, participation in the immune response, blood clearance of particulate matter and foreign substances, lipid metabolism, iron metabolism, steroid metabolism, participation in control of microcirculation and hemostasis) attributed to RE cells, phagocytosis has been the most frequently studied, and serves as a quantitative assessment of RE cell function.

During the past 15 years, a considerable body of evidence has accumulated, in both animals and man, which suggests that RES phagocytic indices (i.e., K values or half-times of clearance, amount of opsonic α_2-glycoprotein) may provide a diagnostic and prognostic indicator of the shock syndrome and its response to therapy (see references 3, 6-8, 11, for recent reviews). Irrespective of the form of shock or its etiology, the magnitude of the RES phagocytic index parallels the degree of shock or trauma; i.e., the greater the degree of injury or shock, the lower the K value. Subjects that eventually die from circulatory shock exhibit depressed RE systems up until death. In marked contrast to what happens in non-survivors, subjects that survive these lethal shock procedures show, with time, progressively improved RES phagocytic indices. Furthermore, usually within 24-96 hr, such subjects exhibit K values that are increased by more than 100% over normal control levels. These hyperfunctional RE systems usually return spontaneously to normal within 96-124 hr. K values and/or the amount of recoverable opsonic α_2-glycoprotein thus appear to reflect, fairly accurately, the degree of tissue injury and to indicate whether a subject will survive or not. The use of K

values and the assessment of circulating, recoverable opsonic α_2-glycoprotein clinically in man, in low-flow states, should be very promising since RE cell phagocytic clearances and assay of opsonic α_2-glycoprotein are relatively safe, can be done sequentially, and are fairly easy to do in man.[15-17]

RES CELL-STIMULANTS IN SHOCK THERAPY VERSUS RES CELL-DEPRESSANTS

At present, RE cell stimulation seems to constitute the only reasonable approach to adapting man to the insult of circulatory shock and trauma prior to his exposure to these body injuries. This could have its greatest value as a pretreatment of patients who, on an elective basis, require one of the massive operative procedures becoming commonplace in cancer surgery, transplantation, and open-heart procedures. The high mortality and morbidity rates associated with these and other types of massive operative procedures could conceivably be reduced by "shock prophylaxis."

In the past, a number of substances and colloids have been shown to stimulate RES phagocytic function in animals (see, e.g., references 3,4,7,8,18,19). A number of these substances (e.g., zymosan, bovine albumin, endotoxin, glucan, restim) were indeed found to be capable of protecting animals against several types of experimental shock and trauma; but these are not suitable for human use, and they are either toxic or non-excretable.[7] If, however, RES-stimulating materials could be found that (a) are not toxic and are excretable (metabolized), (b) yield significant protection against a number of forms of circulatory shock (e.g., hypovolemic, trauma, combined injuries, septic, etc.), and (c) are compatible for human use, then one could use such substances clinically.

It has been reported that when rats and mice are pretreated for only 1 to 3 days with choline chloride, microaggregated denatured human serum albumin, glyceryl trioleate, estrogens, glucocorticoids, or quinones, these agents not only effectively stimulate RES phagocytic activity 125-500% over controls but also enhance survival of rodents exposed to several different types of circulatory shock and trauma.[3,7,8,11,19-30] Although some of these substances, which are all compatible for human use, do not protect against all

types of shock when used singly, combinations of these safe RES stimulants will greatly enhance protection against most types of shock, at least in rats.[7,9]

Materials that block or depress the phagocytic powers of RE cells, in most cases, increase shock mortality. Included among these materials are lipids, antibiotics, drugs, micro-organisms, bacterial by-products, and excess amounts of a variety of colloids (see references 7, 8, 19 for reviews). The experimental data on RES stimulants provide a cogent argument for employing some of these materials prior to elective surgery in patients who either require massive operative procedures or are debilitated (high-risk) and require minor surgical procedures. In addition, experimental data with certain vasoactive drugs and pharmacologic antagonists (see below), which are of therapeutic value in circulatory shock, demonstrate that these agents result in RES phagocytic stimulation within 1 to 3 hours after administration.[3,7,8,11,26-28,31-35] Collectively, such evidence (together with other data below) suggest that the RES may represent the homeostatic system serving as a common pathway in different forms of circulatory shock and trauma.

Is there, then, a possibility that many of these RES-active substances, in protection against the lethal sequelae of agonal events in shock syndromes, may not directly act on RE cells? That is to say, could these RES-stimulating substances be producing enhancement of RES cell phagocytosis via the synthesis and/or release of an intermediary substance(s) from some key organ, tissue, or cell? In addition, is there a connection or relationship between RES function, local regulation of blood flow (i.e. microcirculatory blood flow), and peripheral tissue blood flow in low-flow states?

EVIDENCE THAT RES-STIMULATING SUBSTANCES PRODUCE RES CELL PHAGOCYTOSIS-PROMOTING SUBSTANCES WHICH EFFECT MICROCIRCULATORY STABILITY IN LOW-FLOW STATES

Previously, we demonstrated that rats pretreated with choline chloride (CC) exhibited enhanced RES phagocytic function, increased spleen size, and protection against hemorrhage.[20] In view of these findings, experiments were undertaken with rats to determine whether the spleen plays a role in CC-induced RES phagocytic stimulation and protection against hemorrhage. Although chronic treatment of intact rats with CC produced the latter effects, CC treatment of splenectomized rats failed to result in either a stimulation of RES cell phagocytosis or protection against hemorrhage.[29] Utilizing several other forms of trauma (e.g., intestinal ischemia, Noble-Collip drum trauma), we have recently obtained qualitatively similar results with chronic treatment of rats with aggregated denatured human serum albumin, triglycerides, estrogens, and certain quinones; i.e., removal of the spleen greatly attenuated the RES cell hyperphagocytic events and the protection against shock and trauma (unpublished findings). We believe that such findings point to a role for some types of splenic cell(s) in both RES cell stimulation and shock protection. Since splenic extracts from trauma-resistant animals have previously been shown to confer protection to naive rats and mice against shock and trauma,[24,29] there is a real possibility that a common substance(s) is being generated in the spleen, by the action of a number of these RES cell stimulants on certain cell types. One can then envision that the active substance(s), after being released from the spleen, would then be free to promote phagocytosis and act on the microcirculation. These tenets are now being tested in our laboratory. Since the liver, or the Kupffer cells, were not observed to hypertrophy with our RES cell stimulants, it must be entertained that the unknown released substances could affect microcirculatory stability, i.e., prevent decompensatory reactions (Table 1) from taking place after trauma, hypovolemia, sepsis, etc.

TABLE 1. PROGRESSIVE HEMODYNAMIC PHASES OF SHOCK SYNDROMES

Progressive Phase

Compensatory

Ischemic hypoxia
Reflex arterial, precapillary, and arteriolar vasoconstriction
Arteriolar-venular shunting
Venous vasoconstriction

Stagnant Hypoxia

Gradual fading of precapillary and arteriolar vasoconstriction
Sustained venular constriction
Progressive decline in venous return
Progressive fall in arterial blood pressure
Fall in cardiac output

Decompensatory (Refractory; Irreversible)

Arteriolar and precapillary vasodilation
Venular dilation and atony
Capillary and postcapillary venular pooling
Extravascular shifts of fluid
Microembolism
Progressive fall in arterial blood pressure and cardiac output

If the latter statement has validity, then RES-stimulating substances (such as microaggregated denatured human serum albumin), which do not have vasoactive properties per se,[21,22] might be expected to prevent the loss of venular tone and venous return noted in the agonal stages of circulatory shock (Table 1). In other words, such RES cell stimulants should indirectly (via synthesis and release of unknown mediators) result in beneficial actions on microvessels and/or microvascular tone. The summary data shown in Table 2 do indeed indicate that RES cell stimulation with microaggregated denatured human serum albumin in rats resulted in a maintenance of venular tone. Similar findings have been noted, so far, with choline chloride as well.[8,11] Such experiments suggest that RES cell stimulants are probably resulting in a synthesis and release of vasoactive substances that can play a role, i.e., stimulate RES cells and alter microvascular tone.

TABLE 2. INFLUENCE OF COLLOIDAL ALBUMIN PRETREATMENT ON SURVIVAL, RES PHAGOCYTIC INDICES, AND VENULAR TONE AFTER BOWEL ISCHEMIA AND TRAUMATIC SHOCK[a]

Group	Survivors/Total	RES Index (K; mean + S.E.)	Venular Lumen (μm + S.E.)
Controls	20/20	0.053 ± 0.002	38.5 ± 2.5
Bowel Ischemia			
Saline	2/20	0.009 ± 0.003[b]	49.6 ± 4.2[b]
Albumin	16/20	0.043 ± 0.004[c]	40.2 ± 2.8[c]
Trauma			
Saline	4/20	0.016 ± 0.002[b]	47.4 ± 3.1[b]
Albumin	10/20	0.032 ± 0.005[c]	41.5 ± 2.9[c]

[a]Colloidal denatured human serum aggregate albumin (100-200 A diam), 21.6 mg/kg i.v., 2 times/day for 3 days. Animals subjected to shock on 4th day.
[b]Significantly different from controls ($P < 0.01$).
[c]Significantly different from controls and saline (shocked) animals ($P < 0.01$).

REGULATION OF BLOOD FLOW, VASOACTIVE MEDIATORS, AND RES PHAGOCYTIC FUNCTION

Phagocytic uptake of particulate matter and microorganisms by RE cells, especially those in shock-target organs (e.g., liver, spleen, lungs, and lymph nodes), is dependent upon an optimal local blood flow. It is known that local control of blood flow in different tissues is dependent upon a balance between endogenous constrictor and dilator substances and metabolites.[36-38] The fact that a number of these biologically active materials with vasotropic effects appear in the tissues and blood in shock (Table 3), particularly when the organisms becomes refractory to therapy, suggests that the final functional deterioration of the cardiovascular system may be due to the specific action of one or more of these biologically active materials. It has been suggested that such substances affect RE cell phagocytosis. Evidence has been presented demonstrating that acute infusion of a number of these vasoactive

(endogenous) agents results, within 3-24 hr, in RES depression or stimulation, depending upon substance.[7] One might therefore ask whether or not small quantities of these neurohumoral substances and/or metabolites, which are normally present in the circulation, play fundamental roles in regulating (conferring a tone on) phagocytic processes of fixed RE cells. On the other hand, the emission of excessive quantities of these substances, on the other hand, as appears in shock, could exert deleterious (depressant) actions on RE cells.

Many changes could account for the elaboration of such biologically vasoactive materials, e.g., (a) tissue hypoxia on a systemic or local level; (b) cellular destruction from ischemia and trauma; (c) altered blood coagulation; (d) activation of proteolytic enzymes; (e) hormonal (neurotransmitter) discharges; (f) changes in metabolic by-products (e.g., CO_2, O_2, H^+, etc.); and (g) failure of RE cells, per se, to detoxify these biologically active substances.

TABLE 3. VASOACTIVE MEDIATORS RELEASED INTO BLOOD IN SHOCK AND LOW-FLOW STATE SYNDROME

Amines

- Histamines
- Serotonin
- Catecholamines
- Acetylcholine

Peptides

- Kinins
- Angiotensins
- Vasopressin
- Substance P
- MDFs*
- G.I. Tract peptides
- Enkephalins, endorphins
- Neuropeptides?

Fatty Acid Derivatives

- Prostaglandins
- Thromboxanes
- Endoperoxides
- Prostacyclin
- Lipoxygenase products

Metabolites

- Lactic acid
- Glycolytic intermediates
- Adenyl compounds

Purines

- Adenosine

* Myocardial depressant factors

Excessive concentrations of these highly active chemicals (Table 3), particularly when their concentrations are disproportionate in relation to each other, certainly must result in an imbalance in many specific homeostatic mechanisms, which may then account for some of the total systemic and regional dysfunction in shock. Since so many of the body's homeostatic mechanisms are regulated on the feedback principle, it becomes understandable that these abnormal materials released in circulatory shock can in turn alter the neurogenic and humoral over-activity that released them.

MAST CELL RELEASE OF HISTAMINE PRODUCES LETHALITY AND TOLERANCE IN SHOCK: RELATIONSHIP TO RETICULOENDOTHELIAL SYSTEM FUNCTION

Systemic administration of compound 48/80, a condensation product of p-methoxy-N-methyl-phenethylamine and formaldehyde, into a variety of mammals is known to rapidly lower arterial blood pressure and produce circulatory shock.[39] Although it is known that systemic injection of compound 48/80 results in a potent liberation of histamine from mast cells,[39] pretreatment with antihistamines does not always result in protection against the lethal actions of 48/80.[40] Moreover, there are a number of in vivo observations that indicate that if antihistamines do exert protection, this may not be related to the "true" pharmacologic antagonism of these substances against histamine.[40] It is interesting to note that injection of small but increasing amounts of compound 48/80 into rodents can produce tolerance to LD_{100} doses of this compound.[41] In addition, such treated animals become cross-tolerant to endotoxin shock; the mechanism(s) of this tolerance is, however, not known.

In view of the importance of RE cells to host defense, and the dilemma as to how compound 48/80 produces shock and tolerance to circulatory stress, we decided to examine: (a) whether 48/80 produces concentration-dependent effects on shock lethality, (b) whether the latter was related to the RES phagocytic functional state, and (c) whether animals made tolerant to lethal doses of 48/80 were cross-tolerant to lethal whole-body trauma and whether such animals would demonstrate enhanced RES phagocytic function.[42]

Table 4 indicates the survival rates and RES phagocytic indices for animals receiving varying acute doses of compound 48-80 together with those of saline-treated animals. It is apparent from the K values (determined 30 min after 48/80 i.v.) that the greater the dose of compound 48/80 administered, the greater is the magnitude of early RES phagocytic depression, and the lower is the survival rate. In contrast, animals given 48/80 in increasing doses for 7 consecutive days exhibit a hyperphagocytic RES (almost four fold over saline controls) and are completely resistant to an LD_{90} episode of whole-body trauma (Table 5). Such animals are also completely resistant to the lethal effects of an LD_{100} dose (i.e., 2.5 mg/kg) of compound 48/80 (Table 5).[42]

TABLE 4. ACUTE ADMINISTRATION OF COMPOUND 48/80 INFLUENCES RETICULOENDOTHELIAL PHAGOCYTIC FUNCTION AND SURVIVAL IN RATS

Treatment	Dose (mg/kg)	RES Phagocytic index (mean ± S.E.M.)	% Survival
Saline	-	0.052 ± 0.002 (38)[a]	100 (20/20)[b]
48/80	0.05	0.042 ± 0.004[c] (12)	100 (14/14)
	0.10	0.038 ± 0.003[c] (12)	80[c] (8/10)
	0.20	0.016 ± 0.002[d] (14)	30[c] (6/20)
	0.30	0.008 ± 0.002[d] (12)	10[c] (2/20)
	1.0	0.003 ± 0.001[d] (10)	5[c] (1/20)
	2.5	0.001 ± 0.001[d] (10)	0[c] (0/20)

[a]Numbers of different animals.
[b]No. survivors/total rats.
[c]Significantly different from saline controls ($P < 0.001$).
[d]Significantly different from all other values ($P < 0.001$).

TABLE 5. REPEATED ADMINISTRATION OF COMPOUND 48/80 ENHANCES RES PHAGOCYTIC FUNCTION AND RESISTANCE OF RATS TO WHOLE-BODY TRAUMA

Treatment	RES phagocytic index (mean + S.E.M.)	% Survival
Saline	0.049 ± 0.003 (18)[a]	100 (15/15)
Repeated 48/80	0.182 ± 0.014[c] (14)	100[b] (12/12)
NCDT-trauma[d]	0.003 ± 0.001[e] (12)	10 (1/10)
Repeated 48/80 + NCDT-trauma	0.155 ± 0.015[c] (12)	100 (15/15)

Compound 48/80 was administered IV in the following doses: 50 µg/100, 1st day; 100 µg/100 g, 2nd day; 200 µg/100 g, 3rd day; and 300 µg/100 g, 4th-7th days

[a]Numbers of different animals
[b]Challenged with LD 200 dose (2.5 mg/kg) of compound 48/80 IV
[c]Significantly different from saline controls ($P < 0.01$)
[d]Challenged with 850 revolutions of Noble-Collip drum trauma
[e]Significantly different from all other values

This clearly demonstrates that graded anaphylactic shock induced by compound 48/80 produces marked and dose-dependent early phagocytic depression of fixed macrophage cells. The finding that one can make animals completely tolerant to LD_{100} doses of the histamine releaser and mast cell disruptor, compound 48/80, confirms previous reports.[41] That such adapted animals and histamine-depleted animals, exhibit RE systems that are almost 400% stimulated over control levels, and are cross-tolerant to whole-body trauma is of special interest since it supports the notion that RE cell stimulation is involved in host defense against trauma (vide supra). Our findings could be interpreted to indicate that RE cells play a pivotal role in the shock-like and shock-tolerant actions of compound 48/80. Such findings suggested to us that anti-histamines might be efficacious in shock; if so, these histamine receptor blockers should result in RES cell stimulation and stabilization of micro-

circulatory integrity and normalization of venular tone after induction of circulatory shock and/or trauma.

H_1 RECEPTOR ANTIHISTAMINES PROTECT AGAINST SHOCK AND TRAUMA: EVIDENCE FOR ROLE OF HISTAMINE AS A SHOCK TOXIN AND RELATIONSHIP, TO RES FUNCTION AN MICROCIRCULATION

Recently, we have demonstrated that pretreatment of animals with several different histamine H_1-receptor antagonists (e.g., diphenhydramine, chlorpheniramine, promethazine, pyrilamine, pyribenzamine) exerted significant protection against whole-body trauma, hemorrhage, and bowel ischemia shock.[2,43,44] Rats and mice pretreated with H_2-receptor antihistamines (e.g., burimamide, metiamide) demonstrated an exacerbated mortality after induction of trauma or shock. The hemodynamic parameters that were assessed paralleled the beneficial (H_1-antihistamines) and detrimental (H_2-antihistamines) actions of the histamine antagonists.[43]

Our more recent studies indicate that the marked RES phagocytic depression seen early in shocked and traumatized control rats can be completely prevented by pretreating animals with H_1-receptor blockers.[45] Pretreatment with H_2-receptor blockers (i.e., metiamide, burimamide) not only exacerbates mortality but also results in further decrements of RES phagocytic depression to a level below that seen in unpretreated rats.[45]

Considerable evidence has accumulated to suggest that in the early stages of circulatory shock and trauma, the arterioles and small muscular venules in the splanchnic vasculature constrict to compensate for fluid and/or blood loss (see Hershey and Altura, 1973 for review).[46] In view of the latter, we thought it might be useful to determine whether or not the H_1- and H_2-receptor blockers could influence the constrictor response of splanchnic resistance and capacitance vessels in the early stages of circulatory shock.[42] The data in Table 6 concerning rats bled to 35-40 mm Hg) clearly demonstrate that H_1-receptor antihistamines, which protect in shock, prevent microvessels from over-constricting, whereas the H_2-receptor blockers, which exacerbate shock mortality, exacerbate the degrees of arteriolar and venular vasoconstriction; the former thus results in an increase in tissue perfusion and blood-tissue

exchanges, whereas the latter results in further tissue ischemia and cellular damage.

TABLE 6. ARTERIOLAR AND VENULAR LUMEN SIZES 45 MIN POST-HEMORRHAGIC SHOCK IN RAT MESENTERY BEFORE AND AFTER ANTIHISTAMINE TREATMENT

Micro-vessels	N	Control	After Hemor-rhage	After diphen-hydramine (1 mg/kg)	After metiamide (10 mg/kg)
Arterioles	6	23.5 ± 1.2	12.2 ± 0.6[a]	22.8 ± 1.4	4.8 ± 0.4[b]
Muscular	6	46.6 ± 2.8	34.2 ± 1.4[a]	42.8 ± 3.2	24.2 ± 1.1[b]

[a]Significantly different from controls before hemorrhage ($P < 0.01$)
[b]Significantly different from all other values ($P < 0.01$)

Collectively, these results suggest several things: First, histamine-induced vasodilation via H_2-receptors, in shock target-organ regions, may be a beneficial effect in cardiovascular decompensation in circulatory shock and trauma. Second, RE cell phagocytic function parallels the effectiveness of antihistamine shock prophylaxis and therapy. Third, certain actions of endogenously synthesized and released histamine on H_1-receptors in peripheral blood vessels may be detrimental. Fourth, H_1-receptors antihistamines can restore microcirculatory blood flow capillary distribution to normal in circulatory shock. In view of these data, we believe one must think seriously about the values of antihistamines as adjuvant drugs in the treatment of low-flow states and as preoperative medication.

One must now ask whether the specialized cells that line the microvessels (e.g., smooth muscle, endothelial cells) per se could be responsible for an elaboration of histamine and/or other humoral mediators?

EVIDENCE THAT PHARMACOLOGICALLY ACTIVE, FREE HISTAMINE CAN BE SYNTHESIZED AND RELEASED LOCALLY IN THE MICROCIRCULATION: POSSIBLE RELATION TO ENDOTHELIAL CELLS

Many endogenous and tissue-synthesized dilator substances have been suggested to participate in the moment-to-moment regulation of microvascular tone.[36-38,47,48] During the past few years, some information has been brought forth that implicates histamine as a dilator mediator of reactive or postocclusion hyperemia.[49] Very few studies, however, have been performed at the microcirculatory level. Recently we found that the H_2-histamine receptor antagonist, metiamide, could attenuate rather markedly (e.g., 33% to 49% reduction) a postocclusion vasodilator response of single mesenteric arterioles, 20-22 μm i.d. (Table 7).[50] In addition, we have now found that the more potent H_2-receptor antagonist cimetidine, when superfused on rat mesentery, will result in a 52% to 68% reduction in the postocclusion vasodilator response seen in single arterioles, 20-22 μm i.d. (Table 7).[42] It should be noted that administration of metiamide (or cimetidine) did not influence resting arteriolar tone or responsiveness to locally applied PGE_1 or epinephrine. These findings are consistent with the concept that histamine may play some role in hyperemic responses.[51,52] Further, these results suggest that these histamine-induced hyperemic responses are probably mediated via H_2-histamine receptors. In 1935, Barsoum and Smirk reported that, after a period of complete occlusion of the circulation, the venous blood of man contained an increase in the concentration of a substance that had the biologic properties of histamine, thus supporting the original suggestion of Lewis (1927) that an "H" substance may be responsible for reactive hyperemia.[51]

Although it might be argued that our data with metiamide and cimetidine provide evidence for the idea that histamine can be synthesized locally by microvessels (Schayer, 1974) (e.g., in stress and shock[52]), our observations do not provide evidence for the concept that this biogenic amine is synthesized continuously by microvessels. The failure of both metiamide and cimetidine to alter resting tone, at concentrations known to specifically antagonize mesenteric dilator responses to histamine, strongly argues against

the idea of histamine being the sole dilator regulator of the microcirculation. Although the latter may, in the final analysis, have to be modified, our data together with those reported elsewhere,[6,9,10,42] do suggest that histamine can be synthesized locally by either EC or smooth muscle cells, or both.

TABLE 7. INHIBITION OF MESENTERIC ARTERIOLAR POSTOCCLUSION VASODILATATION BY SUPERFUSION WITH H_2-RECEPTOR ANTAGONISTS

Antagonist (μg/min)	N	Arteriolar lumen size (μm ± S.E.M) Control	Post-occulsion response	Post-occulsion response with drug antagonist	% Inhibition with antagonist
Metiamide					
10	5	20.8 ± 1.0	24.5 ± 1.1[a]	23.2 ± 1.3[b]	33
30	6	21.3 ± 0.5	25.4 ± 0.6[a]	23.4 ± 0.6[b]	49
Cimetidine					
2	6	21.3 ± 0.5	24.8 ± 0.5[a]	23.0 ± 0.8[c]	52
10	4	20.4 ± 0.8	24.8 ± 1.2[a]	21.8 ± 0.6[c]	68

[a]Significantly different from controls ($P < 0.02$)
[b]Significantly different from postocclusion vasodilator response ($P < 0.05$)
[c]Significantly different from postocclusion vasodilator response ($P < 0.01$).

IMPLICATION OF ENDOTHELIAL CELLS IN DESTABILIZATION - STABILIZATION OF MICROVASCULAR INTEGRITY IN LOW-FLOW STATES

Since EC line the heart, blood vessels, and capillaries, they must perforce play a critical role in homeostasis and control for tissue-blood exchange. Besides performing important physiologic roles in host defense and as a barrier (Table 8), it is now quite clear that EC play key roles in body metabolism (Table 9). EC damage has now been implicated in several diseases of major clinical importance, for example, diabetes mellitus, primary pulmonary hypertension, hypoxic pulmonary hypertension, thrombosis, acute renal failure, atherosclerosis, arterial spasm, antigen-antibody (anaphylactic) disorders and circulatory.[4,6,9,10,54]

TABLE 8. ENDOTHELIAL CELL ATTRIBUTES

Normal Physiology	Abnormal Physiology
"Wettable" intimal surface	"Non-wettable" intimal surface (sticking of WBCs and platelets)
Hemostasis - blood coagulation	Loss of hemostatic factors (thrombi, thromboses, bleeding)
Protective barrier - normal capillary permeability	Increased capillary permeability (transudation of fluids, plasma)
Synthetic machine	Interference with PG synthesis, basement membrane formation, proteases, other enzymes, surface hormone receptors
Maintenance of vascular tone	Loss of microvascular integrity and function

Since EC not only serve as a protecting barrier but are also active biochemical machines (Table 9), it is obvious that disruption or malfunction of these cells could result in vascular injury, hypotension, and some of the sequelae of events observed in the microvasculature of a host subjected to shock.

There is at present no agreement as to why the lungs, kidneys, and liver are often compromised very early after either blood and fluid loss or sepsis.[5,6] Often after what the clinician feels has been acceptable treatment for a "shocked victim," the patient dies of "shock lung," renal failure, or liver failure. Upon autopsy, one usually notes severe congestion and thrombi formations in these vital organ systems.[5,6,55] Close microscopic inspection, employing electron microscopy, usually reveals that a great many of the EC are either destroyed, transformed, or have undergone morphological changes; surface membranes of these EC usually undergo very early changes in these three organ regions.

TABLE 9. BIOSYNTHETIC MACHINERY, SURFACE PROTEINS AND RECEPTORS IN ENDOTHELIAL CELLS

A. Biochemical Machinery to Synthesize:

- Hemostatic factors
  - Factor VIII antigen
  - Factors V, IX, XII
  - von Willebrand's factor
  - Plasminogen activators and inhibitors
- Thromboplastin
  - Heparin-like molecule
  - Platelet aggregation inhibitor
- Tissue factor
- Prostanoids
  - PGE_2
  - PGF_2
  - PGI_2
  - PG_6 - Keto F_1
- Hydroperoxy acids - lipoxygenase products
- Histidine decarboxylase
- Monoamine oxidase
- Proteases
- Collagens
  - Type I, III, IV
- Basement membrane collagen
- Human glomerular basement membrane
- Fibronectin
- 2-Macroglobulin
- CSP-60

B. Proteins Adherent to EC Surfaces:

- Mucopolysaccharides (glycocalyx)
- Angiotensin converting enzyme
- Fibronolytic factor
- Fibronectin
- AD Pases; ectonucleotidases
- Chemotactic factors
  - 2-Macroglobulin

C. Biochemical Machinery to Degrade:

- Adenine nucleotides
- Prostanoids
- Serotonin
- Catecholamines

D. EC Surface Hormone Receptors for:

- Vasoactive Agents
  - Catecholamines
  - Angiotensin II
  - Serotonin
  - Prostanoids
  - Acetylcholine
  - Kinins
  - Substance P
  - Histamine
  - Arachidonic acid
  - Adenosine
- Steroids
  - Estrogens, progesterone
  - Glucocorticoids
- Lipoproteins
  - LDL, HDL
- Complement $C3_a$
- Insulin
- Heparin, Thrombin
- Proteoglycons
- B-thromboglobulin

Since early in shock and trauma, the peripheral vasculature often overcompensates and produces severe ischemia in the splanchnic tract, liver, and kidneys,[2,5,6,46,55] we wondered whether or not the ability of the microvasculature to respond to endogenously released vasodilator substances (e.g.,

histamine, bradykinin, acetylcholine) might not be compromised due to malfunction of the intimal (EC) lining of the microvessels.[9,10]

Failure of Single Arterioles to Produce Hyperemic or Vasodilator Responses Early After Ischemia, Endotoxemia, and Trauma

Although the normal response of arterioles to temporary occlusion is a postocclusion hyperemia (vide supra, Table 7), the summary data presented in Table 10 indicate that irrespective of the etiology of the ischemia (i.e., bowel-ischemia, endotoxin, or Noble-Collip drum trauma), single mesenteric arterioles of the rat fail to yield an appropriate postocclusion hyperemia. The latter could be related to either the (a) failure of EC to produce a vasodilator substance (vide supra, Tables 7 and 9) or (b) the failure of released vasodilator substances, in response to the occlusion, to act appropriately on the microvascular smooth muscle cells. Experiments were designed to determine whether exogenously applied vasodilators (which are known to act via EC[9,10]), such as bradykinin, acetylcholine, and substance P, would be able to produce vasodilatation of single arterioles early after induction of circulatory shock and trauma.

TABLE 10. INHIBITION OF MESENTERIC ARTERIOLAR POSTOCCLUSION VASODILATATION IN SMAO, ENDOTOXIC, AND NOBLE-COLLIP DRUM TRAUMA SHOCK[a]

		Arteriolar Lumen Size (μm ± S.E.M.)			
Type of Shock	N	Control	Control Postocculsion Response	Postocclusion[b] Response After Shock	Percent Inhibition
SMAO	5	20.6 ± 1.2	24.8 ± 1.4[c]	21.2 ± 1.0[c]	86
Endotoxic	6	22.4 ± 1.4	26.9 ± 1.5[c]	20.8 ± 1.6[c]	100
NCDT	4	20.2 ± 1.6	24.6 ± 1.4[c]	21.4 ± 1.8[c]	73

[a]Temporary occlusion of superior mesenteric artery (SMA) for 60 min; LD_{50} IV injection of purified Salmonella enteritidis endotoxin (No. 12047, Difco Labs.); NCDT = 600 revolutions at 40 rpm
[b]Studied 60-75 min post-release of SMA ligature, post IV injection of endotoxin, and post NCDT
[c]Significantly different from control post-occulsion response ($p < 0.02$).

The data shown in Table 11 clearly indicate that irrespective of the ischemic or traumatic etiology, none of four vasodilators was able to produce much in the way of arteriolar vasodilatation early after induction of bowel-ischemia, endotoxemia, or Noble-Collip drum trauma. If the compromised EC were indeed responsible for the failure to elaborate active mediators[6,9,10] in response to the vasodilators, then one could also anticipate that bradykinin, substance P, and acetylcholine (substances that normally modulate or attenuate the actions of neurochemical substances in the microvasculature[36-38,47,48]) might not be able to attenuate the arteriolar constrictor actions of norepinephrine and vasopressin. That the latter is indeed so can be seen from the data presented in Table 12. These direct in vivo quantitative TV microscopic studies clearly revealed that the ability of acetylcholine and bradykinin to attenuate the constrictor effects of both norepinephrine and vasopressin were sharply curtailed early after bowel ischemia.

TABLE 11. SMAO, ENDOTOXIC AND NCDT SHOCK RESULT IN MARKED HYPORESPONSIVENESS OF MESENTERIC METARTERIOLES OF MALE RATS TO LOCAL ADMINISTRATION OF VASODILATOR AGENTS[a]

Vasodilator (dose, μg)	N	% Increase in Lumen Size[b]			
		Controls	SMAO	Endotoxic	NCDT
Acetylcholine (1.0)	3-6	37	18	9	15
Histamine (1.0)	4-7	46	21	15	17
Bradykinin (0.01)	3-6	26	7	4	10
Substance P (0.1)	3-4	22	5	3	6

[a]Vasodilator agents were applied topically (0.1-ml volumes) before and 30-45 min post SMAO, endotoxic (LD_{50} S. enteritidis) and NCDT shock.
[b]All experimental values are significantly different from controls ($P < 0.01$).

TABLE 12. FAILURE OF INTRA-ARTERIAL ADMINISTRATION OF ACETYLCHOLINE OR BRADYKININ TO ATTENUATE RAT MESENTERIC ARTERIOLAR CONSTRICTIONS INDUCED BY NOREPINEPHRINE AND ARGININE-VASOPRESSIN AFTER SMAO SHOCK

Constrictor Agent, Dose	N	% Attenuation of Constrictor Response			
		Before Shock		SMAO Shock	
		Ach[a]	Bk	Ach	Bk
Norepinephrine (1.0 nm)	5	40	60	5[b]	8[b]
Vasopressin (1.0×10^{-4} nM)	4	15	12	0[b]	0[b]

[a]Acetylcholine (3 µg/min) or bradykinin (0.1 µg/min) was administered via branch of ileocolic artery before and 45-60 min post SMAO shock at least 10 min prior to topical application of norepinephrine or vasopressin.
[b]Significantly different from control (before shock) observations ($P < 0.01$).

Overall, we believe the data presented and summarized in Tables 10-12 constitute presumptive evidence for the notion that an inability of released vasodilator substances to modulate the compensatory peripheral vasoconstriction may be a pivotal mechanism for why the host often is difficult to treat and goes on to die. If the latter rationale is correct, then normal pulmonary and renal vessels selectively denuded of their inner endothelial layer should fail to relax in response to vasodilators, such as bradykinin and acetylcholine.

SELECTIVE REMOVAL OF ENDOTHELIAL CELLS RESULTS IN THE INABILITY OF RENAL AND PULMONARY VESSELS TC RESPOND TO BRADYKININ AND ACETYLCHOLINE

During the past 4 years, we have demonstrated that vascular EC of intrapulmonary, intrarenal, and hepatic arteries appear to play an obligatory role in relaxation of these blood vessels to several different types of naturally occurring (and released) vasodilators, e.g., acetylcholine, bradykinin, arachidonic acid, substance P, ADP, and ATP (e.g., Figures 1 and 2).[6,9,10,57-60] Similar findings have been observed by others as well on a

large variety of vascular smooth muscle types.[6,61-63] It is also clear that these vasorelaxants act on distinct EC receptors (e.g., Figure 3).[6,9,10,54,57-63]

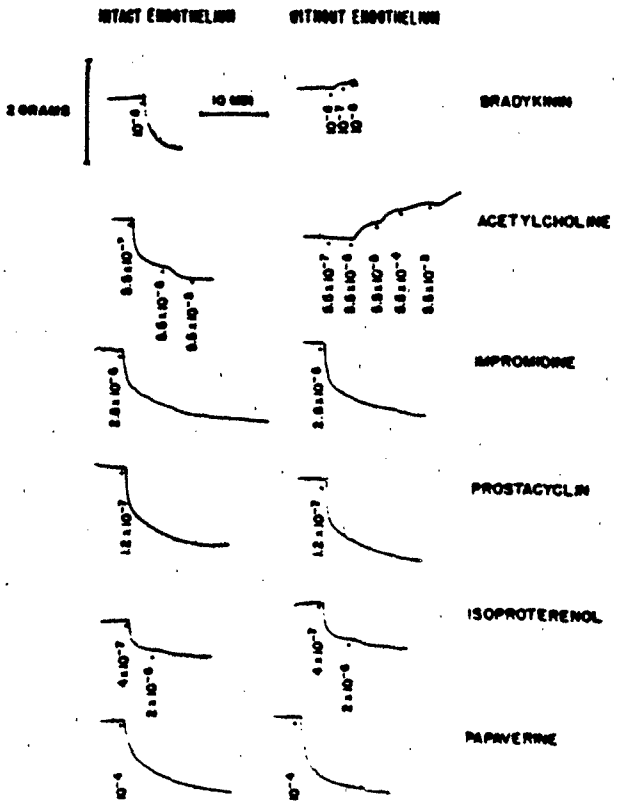


Figure 1. Comparative responses of canine intrapulmonary arteries contracted with serotonin (2.6×10^{-8} to 1.3×10^{-7}M) to bradykinin, acetylcholine, impromidine, PGI_2, isoproterenol, and papaverine in the presence (left) and absence (right) of endothelium. Dots indicate points at which cumulative concentration (moles per liter) of agonist was added. Selective removal of endothelium resulted in a complete loss of relaxant responses to acetylcholine and bradykinin, whereas relaxant responses to other agonists were not altered by selective destruction of endothelium (from Science, ref. 9, with permission of the American Association for the Advancement of Science).

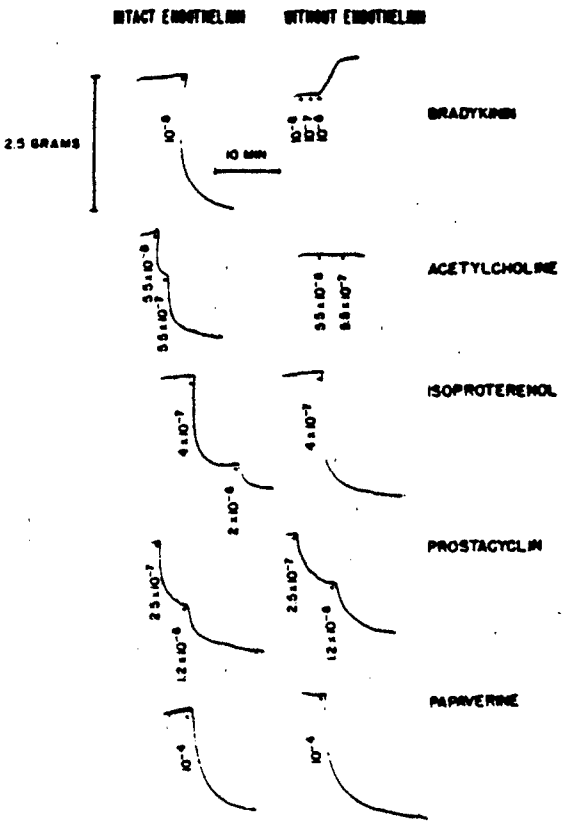


Figure 2. Comparative responses of isolated canine renal arteries contracted by serotonin to bradykinin, acetylcholine, isoproterenol, prostacyclin, and papaverine in the presence (left) and absence of endothelium (right). Note that selective removal of endothelium (right) resulted in a selective, complete loss of relaxant responses to bradykinin and acetylcholine; such a loss in endothelium transformed bradykinin-induced relaxations into contractions. (Taken from Microcirculation, ref. 10, with permission.)

Other relaxants such as isoproterenol (β-adrenergic agonists), prostanoids, papaverine, glyceryl trinitrite, sodium nitrate, sodium nitrite, sodium azide, 5-AMP, and adenosine do not appear to be dependent on the integrity of the endothelium, at least on those blood vessels so far investigated.[6,9,10,54,57-63]

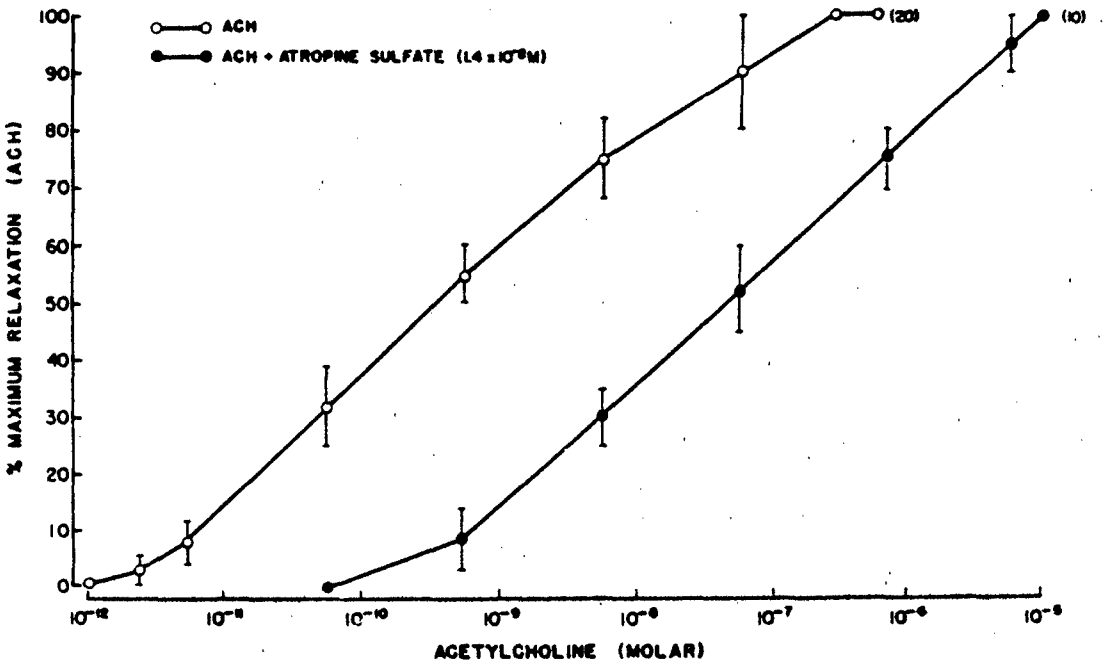


Figure 3. Incubation of isolated canine renal arteries (containing endothelium) with atropine (1.4×10^{-7}M) resulted in a rightward shift of relaxant doses of acetylcholine (ACH) to 50- to 100-fold higher concentration. This demonstrates that atropine acts as a competitive antagonist of ACH on renal arteries exhibiting an intact endothelial lining. (Taken from Microcirculation, ref. 10, with permission.)

If intrapulmonary, intrarenal, intrahepatic, intracerebral, and splanchnic arteries and arterioles suddenly or gradually lose their ability to dilate in situ during circulatory shock, ischemia, or trauma in response to acetylcholine, kinins, adenosine phosphates, substance P, histamine, etc., the end result could be over-vascular compensation and a loss of vascular patency in the organ regions, which could eventuate in parenchymal cell destruction and death. Since those same blood vessels also possess receptors that subserve contraction to these same neurohumoral substances,[6,9,10,35,37,57] the contractile responses would be unmasked if the endothelium were damaged (e.g.,

Figures 1 and 2), thus exacerbating the compensatory constriction brought about by other released neurohumoral agents (i.e., catecholamines, vasopressin, angiotensin II, serotonin, etc.). The end result would be a severe reduction in lumen sizes of the resistance and capacitance vessels in the lung ("shock lung"), kidneys ("renal failure"), liver ("hepatic failure"), brain ("cerebral ischemia") and splanchnic tract, producing a severe (multiple-organ) ischemia, and leading to the eventual sequelae of events noted clinically in shock and on autopsy. A loss of EC integrity in circulatory shock and trauma could also be directly responsible for (a) the inability of patients to respond appropriately to vasodilator therapy and (b) the fact that such treated patients sometimes become worse on administration of certain so-called vasodilator agents.

RELATIONSHIP OF EC DAMAGE TO RES PHAGOCYTIC FUNCTION AND MICROVASCULAR INTEGRITY IN LOW-FLOW STATES

Since all of the RES cells are modified EC, and since EC damage would result in loss of blood flow to the RES cells, one must consider the possibility that RES cell phagocytic function would be compromised early after shock, ischemia and trauma, exactly as has been noted herein (vide supra) and elsewhere.[2,3,6-8,11,15-19,22,26-28,30-35,42,45,56] Graded damage to EC would also thus be able to explain, in part, why the greater the injury or trauma is to the host, the greater is the loss of RES phagocytic capacity.[3,7,11,26,64-66] Moreover, EC damage coupled with failure of RES phagocytic function would feed back in a positive manner to produce further microcirculatory ischemia and damage, thus leading to what is so often termed in shock and trauma "a vicious cycle," which is self-perpetuating and leads to eventual demise of the host. This scheme can be envisioned conceptually by examining these hypothetical events as outlined in Figure 4.

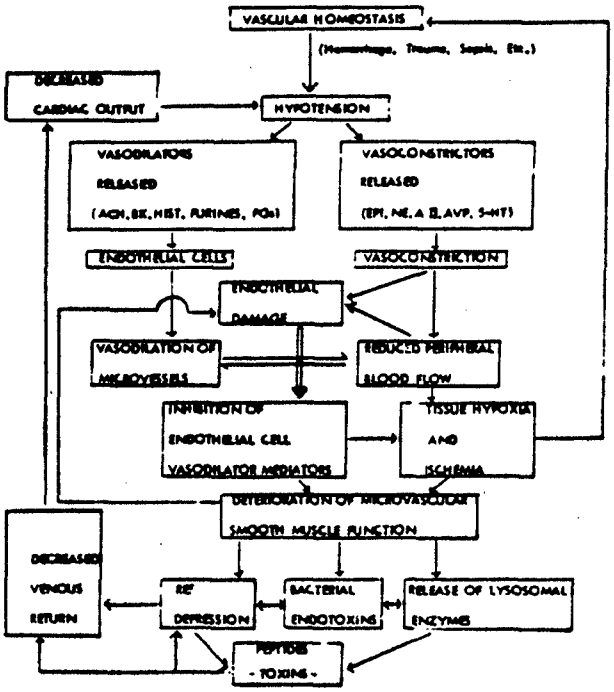


Figure 4. Hypothetical hemodynamic sequence in circulatory shock and proposed site of endothelial cell mediation

The early vulnerability of certain organ regions (e.g., lungs, kidneys, liver, and brain) to decreased tissue perfusion in shock and trauma states, and leading often to "shock lung," renal failure, liver failure, and cerebral infarctions, respectively, may be due to the vulnerability of vascular EC and EC surfaces to hypoxia-induced injury. Denudation or injury of normal pulmonary, renal, hepatic, and cerebral vascular EC would prevent circulating neurohumoral substances (e.g., acetylcholine, bradykinin, arachidonic acid, substance P, etc.) from eliciting vasodilation. Such endothelial injury could transform these circulating and released vasodilator substances into powerful constrictor agents, the end result of which could be severe tissue ischemia, "shock lung," renal failure, liver failure, and cerebral infarctions. Prevention of such EC injury early in circulatory shock and early after trauma could form the basis for a new therapeutic approach in low-flow state syndromes. Some of the RES cell stimulants reviewed above, together with the neuroendocrine and RES cell stimulants discussed elsewhere[3,7,11] appear to be useful and logical approaches toward the latter end.

Overall, the data acquired so far suggest that the phagocytic cells of the RES in some way can alter microcirculatory function, integrity, and blood flow (vide supra, Figure 4). In this context, beneficial pharmacologic manipulation of the microcirculatory apparatus in shock states enhances RES cell phagocytic function as well as survival.[2,3,6-8,11,26,27,28,30-35,45,46]

The material presented and reviewed herein proposes rational hemodynamic therapeutic approaches toward manipulation of the deranged microcirculatory apparatus in low-flow states. At this juncture in time, the outlook for effective and precise pharmacological manipulation of the cardiovascular system, via actions on RES cells and EC, is very much alive and optimistic. We believe that precise insights into the latter will result in effective treatment of refractory shock.

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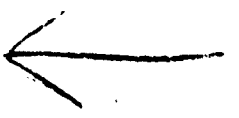
DISCUSSION PERIOD WITH DR. ALTURA

DR. FRANK: Could you describe how you remove endothelial cells?

DR. ALTURA: After removing a vascular strip, filter paper is rubbed gently over the lining for 30-60 seconds which removes the endothelial surface. One could do the same thing in an intact blood vessel by taking a rod and putting it through the intima, and turning it a few times will result in the same denudation of endothelial surface.

DR. ULEVITCH: You mentioned the synthesis of bradykinin by endothelial cells. Could you elaborate on that.

DR. ALTURA: What I should have said is that the enzymes responsible for the sequelae of events, that is those that act on the alpha-2 globulin fraction in the blood, have been found in the endothelial cells.



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DISTURBANCES IN FIBRONECTIN AFTER TRAUMA: RELATIONSHIP TO ALTERED RETICULOENDOTHELIAL FUNCTION MECHANISMS

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INTRODUCTION

Traumatic injury and its sequelae, which many times consist of infection or sepsis and multiple organ dysfunction, are a major medical problem in civilian practice and military medicine. Although the literature concerning the physiopathology of trauma is voluminous, our current understanding is very poor concerning what causes some patients to develop post-traumatic morbidity and mortality while other patients seem to tolerate a similar degree of injury without significant post-traumatic complications. One important aspect of the post-traumatic resistance includes (a) the ability of host defense processes to protect the organism from endogenously generated toxic substances and (b) the protection of the organism from exogenous bacterial infection and other toxic substances. The research carried out in our laboratory over the last dozen years has investigated the participation of the reticuloendothelial system in host defense processes that are involved in pathophysiological reactions to traumatic injury and shock.

RETICULOENDOTHELIAL SYSTEM

The reticuloendothelial system is defined as a system of mononuclear cells that are characterized by their intense phagocytic capabilities.[1,2] This system of fixed and mobile macrophages is involved in many different physiological processes such as the metabolism of lipid, bilirubin, iron, and protein;[3] participation in the development of cellular and humoral immunity[4] as well as non-specific host defense processes against tumor growth,[5] trauma,[6] shock,[7] and bacterial invasion.[8] The major portion of the RES consists of the sessile macrophages located in the capillary beds of the liver, spleen, lung, and bone marrow, which are by necessity in direct contact with the circulating blood. The hepatic macrophages, or Kupffer cells, which line the sinusoids of

the liver, because of their availability to a high proportion of the cardiac output, contribute a major part in the intravasculature clearance of circulating particulates.[8] The importance of the RES in host resistance to shock and trauma was originally demonstrated by work performed by Zweifach's group[7] and Fine's group.[9] These studies were performed using the colloid clearance technique, which can quantitate RES clearance function by assessing the kinetics of the removal of an injected dose of colloidal material, usually gelatin-stabilized.[10] Animals demonstrating enhanced RES function, or hyperphagocytosis, showed an enhanced rate of removal of particulates from the circulation while animals demonstrating depressed RES function, or hypophagocytosis, showed a decreased rate of removal of particulates from the circulation.

Since the colloidal material that is removed from the circulation is located in the RE cells, this method can be used to quantitatively evaluate the activity of this important host defense system. These classic studies demonstrated that experimental injury results in a declining RES activity that is proportional to the severity of the injury. Even more enlightening were studies that demonstrated that the induction of hypophagocytosis results in enhanced sensitivity to experimental injury, while hyperphagocytosis is associated with enhanced resistance to shock and trauma. Thus, the physiological importance of the RES as a major component of "systemic host defense" mechanisms against the consequences of traumatic injury was realized.[8]

These observations stimulated interest in understanding the factors that control RES phagocytic function. Experimental evaluation of macrophage activity as assessed by the vascular clearance of inert or viable particulate substances has demonstrated that RES clearance function is a very complex process that is influenced by numerous factors. Some of these factors include liver blood flow,[11] metabolic status and activity of the macrophage,[12] and the presence of circulating factors in the plasma, which aid macrophage recognition of circulating particulate substances.[8] In other words, the harmful or potentially harmful material in the circulation must be able to be delivered to the cells of the RES. The cells must be able to recognize the particulate, and, once recognized, the cells must be able to phagocytically remove and metabolize or detoxify the ingested substances.

While RE organ blood flow and basal phagocytic and metabolic activity of the macrophage are important factors in controlling RE clearance function,

recent investigations have stressed a critical role of plasma factors in the etiology of RES dysfunction following traumatic injury.[13-15]. The concept that serum factors may be important in phagocytic recognition of particulate matter, especially bacteria, was first postulated in 1895 by Denys and LeClef[16] and demonstrated by Wright and Douglas[17] a few years later. These early investigators were likely studying what we now consider to be specific opsonic factors, i.e., immunoglobulin and complement with respect to phagocytosis. The extension of these studies to non-bacterial particles and thus to the concept of non-specific opsonic substances, was due to the demonstration by Fenn that phagocytosis of carbon and quartz particles was stimulated in plasma.[18,19] Further support for the presence of non-specific or aspecific opsonic factors in the circulation have since been verified in a wide variety of studies with many different "inert" particles. That circulating humoral factors were important in RES recognition of foreign particulate matter was demonstrated by investigating the phenomenon of RES colloid blockade. Colloid blockade refers to the hypophagocytic state induced by the injection of colloidal material into the circulation. Although originally interpreted as being caused by saturation of cells of the RES,[20] subsequent studies revealed that the colloid blockade was, in part, due to the consumption of opsonic factors from the circulation.[21] The realization that RES dysfunction could reflect a depression in the humoral support for macrophage recognition of toxic or potentially toxic substances, led to investigations to determine if alterations in RE function observed after traumatic injury were reflective of alterations in circulating opsonic activity toward RE test colloids.

These studies revealed that, similar to the RES dysfunction observed after colloid blockade, the RES depression following experimental multiple trauma (Noble-Colip Drum Trauma),[13] surgical trauma,[22] hemorrhagic shock,[23] and burn injury[24] was associated with a deficit in the humoral support of hematic macrophage uptake of test colloid, while restoration of normal RES clearance capacity has been associated in all cases with a restoration of this humoral mechanism. These experimental studies in animals thus support the hypothesis that RES dysfunction following trauma is mediated, at least in part, by a deficit in the ability of plasma to support macrophage uptake of RE test colloids. Evidence was provided that this loss or decrease in plasma opsonic activity following traumatic injury may be important in host resistance to post-traumatic sepsis and multiple organ failure. It was provided by clinical

studies demonstrating that in a group of trauma patients who manifested a deficit in plasma opsonic activity, mortality immediately following injury was associated with a sustained deficit in plasma opsonic activity. In contrast, those patients who survived their injury demonstrated normalization of their plasma support for RE function.[25]

FIBRONECTIN IN RES FUNCTION

The demonstration that the depression in RES clearance function following trauma and traumatic injury was in part due to the depression in the humoral support for macrophage uptake of RE test colloids stresses the importance of determining the factor(s) in plasma that is responsible for the opsonic activity. Isolation, purification, and characterization of the protein present in plasma responsible for RES recognition of gelatinized RE test particulate matter demonstrated that this important serum opsonin was a non-immunoglobulin, non-complement-related glycoprotein of MR = 440,000 with an electrophoretic mobility of an alpha-2-globulin.[26] Further biochemical analysis demonstrated that the plasma opsonin was a dimeric protein consisting of identical or nearly identical disulfide linked subunits, each of 220,000 daltons. Immunochemical quantitation of the protein in rat and human plasma revealed that rat plasma[27] contains 400-600 ug/ml while human protein contains 300 ± 25 ug/ml of protein. The RES dysfunction in rats following colloid blockade or traumatic injury was associated with a deficit in the circulating levels of the protein, confirming that such RE dysfunction was due to the consumption of the circulating opsonin rather than the release of an inhibitor into the circulation.

Other studies demonstrated that the intravenous injection of antibody to the opsonic protein sensitized experimental animals to traumatic injury,[14] while the infusion of anti-albumin had no effect, further supporting the hypothesis that the maintenance of humoral support for macrophage function was important in the pathophysiologic response to traumatic injury and sepsis. Additionally, the intravenous infusion of the purified protein reversed the RES dysfunction associated with surgical trauma.[28]

All these studies suggested to us that since post-traumatic sepsis and multiple organ failure, as observed clinically, were associated with a deficit in the plasma opsonic activity as mediated by the opsonic protein (referred to by such names as a specific opsonin, humoral recognition factor, and

alpha-2-opsonic surface-binding (SB) glycoprotein), significant clinical benefit might be achieved by the therapeutic administration of the protein to humans. It can therefore be appreciated how exciting it was to learn that the opsonic protein we had been working with was identical to plasma cold-insoluble globulin (CIg).[29,35]

Cold-insoluble globulin was initially described by Morrison, Edsall, and Miller as a constituent of human plasma that was more insoluble than fibrinogen.[30] This protein, which has been described as a beta-globulin and called cold-insoluble globulin to denote its low solubility at 0°, could not be completely purified from contaminating fibrinogen by these investigators. Initially considered to be somehow related to fibrinogen, it was subsequently identified as a distinct plasma protein. Purification and characterization of human CIg was finally accomplished by Mosesson and Umfleet.[31] It was demonstrated to have a molecular weight of approximately 450,000 and consist of two nearly identical disulfide-linked subunits of 220,000 each.

Considerable interest in this protein was generated when it was demonstrated that the plasma CIg was antigenically related to a fibroblast cell surface protein that was lost during oncogenic transformation in vitro.[32,33] Subsequently demonstrated to be involved in cellular adhesion to inert and collagenous substrata, it was suggested that a more appropriate name for the cellular form of the protein was fibronectin, to denote its function in the adhesion of fibroblasts to solid supports or fibrous connected tissue.[34]

Current convention is to denote the cellular form of the molecule as cell or tissue fibronectin, while the plasma or "soluble" form is designated plasma fibronectin. The demonstration that plasma fibronectin was identical to the plasma opsonin responsible for the augmentation of the macrophage recognition of gelatinized RE test particulates, suggested that the treatment of patients suffering from post-traumatic or post-surgical sepsis and associated multiple organ dysfunction might prove beneficial. Since plasma fibronectin (CIg) was shown to be present in high concentrations in plasma cryoprecipitate prepared by the American Red Cross and used for Factor XIII replacement,[35] therapeutic studies were instituted to determine if the infusion of this plasma fraction to normalize circulating fibronectin levels in this patient population were beneficial.

Initial clinical studies demonstrated that the intravenous administration of 10 units of cryoprecipitate produces a normalization of the plasma opsonic activity.[36] This was associated with an apparent improvement in pulmonary function as judged by a decline in the requirement for positive end-expiratory pressure (PEEP) to maintain adequate blood oxygen tensions. Further improvement in septic status of these patients was judged by enhanced alertness, normalization of blood leukocyte levels, body temperature, blood cultures, and improvement in various hemodynamic parameters. Subsequent physiological studies in a similar group of patients demonstrated an increase in limb blood flow and O_2 consumption, ventilation/perfusion ratio, and renal function after infusion of fibronectin-rich cryo-precipitate.[37] Studies by other groups have confirmed and extended our observations suggesting that the maintenance of adequate levels of circulating fibronectin may be of benefit in the treatment of this type of patient.[38,39]

FIBRONECTIN: ITS POSSIBLE ROLE IN NON-RES HOMEOSTATIC PROCESSES

Recent biochemical studies of the fibronectin molecule have revealed that it is a highly complex molecule possessing binding sites for a variety of biological molecules such as gelatin, collagen, heparin, actin, fibrin, fibrinogen, Clq, DNA, and cells.[40] Consequently, its ability to act as an opsonic substance may be only one of its beneficial properties with respect to the most response to injury. Several studies have suggested that this protein may augment bacterial phagocytosis,[41] while others do not.[42] Recent studies have demonstrated that fibronectin may inhibit fibrin-fibrin interaction, thus preventing the formation of fibrin microthrombi in vivo.[43] It also may participate in the maintenance of the functional integrity of the vascular endothelium, since the administration of fibronectin to sheep (demonstrating increased vascular permeability as the result of septic challenge) depresses pulmonary transvascular protein clearance.[44,45] This observation suggests that fibronectin may help protect the vascular bed from septic injury, thus inhibiting pulmonary interstitial edema so often associated with the septic state.

Fibronectin may also participate in other mechanisms important to host survival after injury. Fibronectin or fibronectin fragments act as chemotractants for fibroblasts,[45] monocytes,[47] and endothelial[46] cells, and

this may participate in mechanisms involved in inflammation and wound repair. It can thus be appreciated that although fibronectin administration following traumatic injury will undoubtedly support increased RE clearance function, the observed improvement in patients may be due to fibronectin's involvement in other important homeostatic processes.

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ISCUSSION PERIOD WITH DR. BLUMENSTOCK

DR. MURANO: The pharmaceutical industry is making an effort in the irection of providing purified fibronectin. So your future studies, perhaps, ight be performed with a more pure preparation.

Your closing comment was that too much fibronectin actually may be etrimental. Are you sure this is due to the fibronectin and not to the xtraordinary amount of fibrinogen amongst other proteins that you are dministering with the cryoprecipitate?

DR. BLUMENSTOCK: It could be that the fibrinogen load in the cryoprecipitate made the patients worse. Perhaps when we give purified fibronectin we may not see that. We have been doing some studies with purified fibronectin in rats to see if we can resuscitate them. We find that when we over-resuscitate them with the purified protein and then test their RE clearance function, the clearance from the blood is very rapid.

DR. MURANO: I believe you made some observation to the effect that the fibronectin may actually be involved in inhibiting the fibrinogen polymerization process. Six to 9 months ago, a group from Poland published a paper in Thrombosis Research, presenting evidence that purified fibronectin will interfere with the polymerization process.

DR. BLUMENSTOCK: Yes, I know that.

DR. MURANO: Do you have any experience with canine fibronectin?

DR. BLUMENSTOCK: Fibronectin is quite easily purified. One of the studies that we have done relates to RE clearance function and how it varies with the fibronectin concentration.

Dogs and other animals have a very rapid clearance function. Dogs have quite high fibronectin levels, between 600 and 800 micrograms per milliliter. The mouse has even more, about 1000 micrograms per milliliter. Man is less than the rat, which is about 400. Sheep have a very low fibronectin level, down around 150 to 200 micrograms per milliliter. It turns out that, if you look at RE clearance in sheep, which have a very small liver, a lot of that material ends up in the lungs. So they may have a little bit different system.

UNKNOWN: At the American Burn Association, Paul Barr presented some data showing a myofibroblast secreting collagen and fibronectin at the tail end, as the cell moved along. He pointed out a very nice role for fibronectin in fibril bundle formation. Could you comment about fibronectin and collagen fibril bundle formation?

DR. BLUMENSTOCK: Fibronectin is being identified with many different properties. Some people, especially those in the tissue culture field, feel that fibronectin actually in many ways is like a hormone and can affect the differentiation of many cell types. If you grow, for example, endothelial cells in very high concentrations of fibronectin, even though they synthesize it, it actually seems they start to form tubes in the culture dish.

On the other hand, fibronectin has been shown to inhibit myotubule formation for myocytes. With respect to a lot of other cell types, it may be a dedifferentiation. I don't know. With regard to the role of fibronectin in cell differentiation, cell movement, and the interaction of cells with collagen, I don't think we even have touched the surface yet.

In wound healing it has been shown that during the initial granulation process there are high concentrations of fibronectin in the wound, and as the wound heals, it is lost. Perhaps fibronectin is involved in the macrophage migration into the wound site and essentially aids in the autodebridement of the wound.

DR. SANTOS: What is the change in fibronectin after whole-body irradiation? If there is downward change, is there a rebound?

DR. BLUMENSTOCK: I am not aware of any studies actually measuring fibronectin following irradiation injury. Studies done by Tom Saba and Nick DiLuzio showed RE depression after whole-body irradiation. Fibronectin, however, was not measured. One of my old professors was relating some work that had been done on irradiated rats. One thing he noticed was a very high alpha-2 globulin level. He had the feeling that this protein was unable to be cleared due to a defect in the macrophages.

There are different forms of fibronectin. We must take that into account when considering the activity of the molecule. For example, during the late periods of tumor growth when RE depression is manifest, there may be lots of fibronectin in the plasma, but it doesn't have any activity.

DP. ULEVITCH: Maybe you could expand a little more on what you just said, because what occurred to me when I saw some of your data was that there were not striking differences in the concentration of fibronectin determined immunologically. I mean, I don't know if it was a 50 percent drop, but you had dramatic decreases in activity.

Have you ever taken plasma from these traumatized rats, isolated that fibronectin, and used that in replacement experiments?

DP BLUMENSTOCK: The answer is no. We are doing that. If you inject gelatin into the system, or collagen, it prevents the antibody from binding to the fibronectin. On the other hand, when actin binds to fibronectin, it does not inhibit the ability of the antibody to measure it, but its activity is depressed. We are redoing those studies in the traumatized man, and we are actually now going to look for actin in the plasma.

Again, it is activity plus amount, not just amount. I think we have to be careful, and so we are redoing all of those studies. It would be nice if all we had to do is measure it, and not have to do bioassays.

AD-P003 868

THE ROLE OF THE MACROPHAGE IN THE HOST RESPONSE TO BACTERIAL ENDOTOXINS

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INTRODUCTION

Gram-negative bacillary infections in man are occurring with increasing frequency in hospitalized patients, and are associated with an unacceptably high mortality rate. In patients with multiple injuries, the likelihood of complications due to Gram-negative infection is high, and despite advances in antibiotic therapy, the prognosis for these patients is bleak. These patients often develop hypotensive shock, disseminated intravascular coagulation, and metabolic abnormalities, all of which are resistant to standard treatment and together contribute to the multiple organ failure observed in these patients. Unique to Gram-negative bacilli is the presence of a complex glycolipid in the outer cell membrane. This component, termed endotoxin or lipopolysaccharide (LPS), is now generally recognized as the bacterial product that is responsible for initiating the biochemical changes leading to shock, DIC, and death.

Despite the early recognition of an important role for LPS in Gram-negative septicemia, it has been difficult to determine the exact mechanisms of injury, in part because of the inability to identify the primary, injurious events occurring after exposure to LPS. In fact, based on *in vitro* studies, we are faced with too many possible mediators since LPS has been shown to activate multiple humoral and cellular mediation systems.[1] Nevertheless, attention has recently been focused on the macrophage as a source of mediators of the early biochemical changes induced by LPS that result in hypotension, DIC, and metabolic changes. The purpose of this report is to describe a number of mediators produced by LPS-treated macrophages that may participate in the host response to LPS, namely, a supernatant factor of LPS-treated macrophage (M), which suppresses adrenocortical steroidogenesis, and a membrane-bound procoagulant activity, which activates coagulation Factor X directly.

SUPPRESSION OF ADRENOCORTICAL STEROIDOGENESIS BY A MACROPHAGE PRODUCT

The therapeutic benefit of steroids in Gram-negative septicemia is controversial.[2,3] Related to this question is the possibility of abnormal adrenocortical function in septic patients.[4] Kelby and Spink,[5] in 1958, suggested that adrenocortical function is normal in patients with bacteremic shock, but since then, a number of other reports have documented conflicting results. Hubay et al.[6] noted patients with adrenocortical insufficiency, Migeon et al.[7] described adrenocortical insufficiency in children with disseminated meningococcemia, and Sibbald et al.[8] reported that 5/26 patients with severe bacteremia demonstrated impaired adrenocortical responsiveness to ACTH. Other evidence for adrenocortical insufficiency derives from the findings of Berry and Smythe,[9] where evidence of decreased adrenal responsiveness to ACTH in mice injected with endotoxin was observed. Finally, the recent study of Keri et al.[10] suggested that soluble mediators might suppress adrenocortical function, since plasma from rabbits in shock as a result of live E. coli infusion suppressed steroidogenesis by ACTH-stimulated, explanted adrenocortical cells. This later study suggested that potential mechanisms of adrenocortical suppression in endotoxic shock should be examined further.

Recently, we examined the possibility that products of LPS-treated M could mediate suppression of adrenal steroidogenesis. Resident and peptone-elicited murine peritoneal macrophages (PEM) were placed in plastic flasks for 2 hr at 37°C in RPMI 1640 with 5 percent fetal calf serum (FCS). After washing to remove the non-adhered cells, one half of the flasks were treated with sufficient macrophage-activating factor (MAF, also known as macrophage cytotoxicity factor) for 4 hr to render the macrophages cytotoxic for tumor cells.[11] The MAF-containing solution was removed, followed by several rinses and replenishment of fresh medium. Then one half of the MAF-treated and untreated cells were exposed to 10 ug/gm Salmonella minnesota Re595 LPS for 18 hr. As controls, flasks without cells were incubated with medium, MAF, and LPS. The macrophage supernatants and control media were then centrifuged, filtered, (0.22 um) and stored at -20°C. Rabbit adrenocortical cells were isolated by collagenase digestion and maintained (as adherent cells) for 3 days in 6-mm-diameter plastic culture dishes in HEPES-buffered MEM with 15 percent FCS. The following additions were made to these adrenocortical cells: (a) 78 ul

MEM-HEPES-FCS; (b) 30 ul macrophage supernatant, control medium, or MEM-HEPES; and (c) 12 ul ACTH dissolved in MEM-HEPES. The final concentration of ACTH in the wells was 10 mU/ml. After 18 hr at 37°C, the adrenocortical supernatants were harvested, and steroids were determined by a fluorometric assay. As shown in Table 1, steroidogenesis was not suppressed by control medium + LPS or by supernatants from macrophages that were not exposed to LPS. However, supernatants from LPS-treated resident and peptone-elicited macrophages did suppress steroid production by approximately 40 percent, and as much as 80-90 percent suppression was observed with supernatants from LPS-treated macrophages that had received prior exposure to MAF. In control experiments we have obtained evidence that (a) the macrophage-supernatant-induced suppression is not a result of LPS carried over from the macrophage culture and (b) the macrophage supernatants do not degrade or inactivate ACTH and do not interfere with the assay of fluorogenic steroids. These results provide support for an LPS-induced macrophage factor(s) that suppresses adrenocortical suppression in

TABLE 1. SUPPRESSION OF ACTH-INDUCED STEROIDOGENESIS BY SUPERNATANTS OF LPS-TREATED PERITONEAL EXUDATE MACROPHAGES[a]

Additions			Fluorogenic Steroid Production (% of maximum response)
Supernatants:			
Macrophage	LK	LPS	
Resident	-	+	59 ± 9
Peptone	-	+	59 ± 11
Resident	+	+	23 ± 5
Peptone	+	+	14 ± 7
Control Sup[b]	±	-	92 ± 9
Control Medium	±	±	98 ± 9
MEM + HEPES + FCS[c]			100

[a] See text for experimental details
[b] Flasks without cells were treated with medium ± LK followed medium ± LPS in the same manner as the macrophage cultures.
[c] Medium used for culture of adrenocortical cells.

Gram-negative sepsis. A number of preliminary experiments have also been performed to characterize the biochemical properties of the M-derived factor. These data are summarized in Table 2. The details of these findings have been recently published.[12]

TABLE 2. PROPERTIES OF MACROPHAGE FACTOR THAT SUPPRESSES ADRENOCORTICAL STEROIDOGENESIS

Treatment	Result
Dialysis using 12,000-14,000 mw cutoff tubing	Activity retained
Ultrafiltration (10,000 mw cutoff)	Activity retained (suppressive activity not observed in ultrafiltrate)
Repeated freeze thawing and storage at -20°C	Stable
Exposure to pH 4 or pH 11 30 min at 37°C	Stable
70°C, 30 min	Stable
100°C, 5 min	Labile
HPLC with Bio-SIL TSK 250	Activity in 40-60 kD range

Studies of the mechanism of ACTH-induced steroidogenesis have provided many details of the molecular basis ACTH action (reviewed in ref. 13). It is known that ACTH binds to plasma membrane receptors, which, as suggested by the recent studies of Buckley and Ramachandran,[14] number approximately 4,000 per cell and have a Kd of 1.4 nM. Calcium and cAMP serve as important second messengers in the response to ACTH. Although a 2-log greater ACTH concentration is required to induce increased cytoplasmic levels of cAMP than is required for induction of steroidogenesis, the dose-response curves for ACTH-induced protein kinase activity and steroidogenesis have been shown to be nearly superimposable.

Since conversion of cholesterol to pregnenolone (the rate-limiting step in steroidogenesis) is blocked by cycloheximide but not actinomycin D, it has been suggested that synthesis of a labile protein is required for cholesterol side chain cleavage to occur. Conversion of pregnenolone to the various steroid products involves, among other things, increased requirement for reducing equivalents (NADPH), a complex array of enzymes that are partitioned in mitochondria and in the smooth endoplasmic reticulum, and mechanisms for shuttling steroid intermediates between the mitochondria and cytosol. We have observed that supernatants from LPS-treated macrophages that suppress the steroidogenic response to ACTH also produce an equivalent degree of suppression of the response to cholera toxin as well as dibutyryl cyclic AMP. Cholera toxin is known to bind to GM1 gangliosides of the plasma membrane with resulting stimulation of adenylate cyclase, whereas dibutyryl cyclic AMP enters the cell and apparently directly stimulates protein kinsase activity. These results suggest that macrophage factor-induced adrenocortical suppression does not result from inactivation of the ACTH receptor or from a block of adenylate cyclase activity, but rather from disruption of steps distal to formation of cAMP.

It has been well appreciated that corticosteroids have marked effects on cells involved in the induction of the immune response and in mediating the inflammatory response. Most recently Snyder and Unanue[15] showed that therapeutic dosages of corticosteroids suppressed interleukin-1 production by LPS-stimulated murine peritoneal exudate cells. Of further interest is the possible connection between cells of the immune and nervous system, suggested by reports demonstrating regulation of the _in vitro_ antibody response by neuroendocrine hormones. Finally, in view of the potential role of endorphins in endotoxic shock, it is clear that previously unappreciated interrelationships between cells of the immune/inflammatory systems and neuroendocrine systems may be of importance in regulating the host response to bacterial endotoxins. The data reported in this paper demonstrating suppression of adrenocortical steroidogenesis by a macrophage-derived product add a new regulatory pathway that may well influence the host response to LPS. Increased understanding of this phenomenon should provide insight into the mechanisms of endotoxic shock.

MACROPHAGE-ASSOCIATED PROCOAGULANT ACTIVITY

After an intravenous LPS injection the liver is the tissue that contains the majority of the tissue-bound LPS.[16] When radiolabled LPS is injected and electron microscopy and autoradiography of tissue sections is performed, LPS localization is observed in the cytoplasm of hepatic macrophages (H-M).[16] Other studies using electron microscopy to identify LPS in tissue sections have described similar findings,[17,18] and thus this M population represents a major cellular target of LPS.

One of the consistent histologic findings following a single injection of LPS is fibrin deposition in the liver microcirculation[19] and association of the fibrin with the H-M (John Mathison, personal communication). These data prompted us to determine if the hepatic M can respond to LPS by producing mediators that might play an important role in LPS-induced fibrin deposition. To accomplish this, we first needed a source of purified H-M, so we used a combination of mechanical and enzymatic disaggregation of rabbit liver to prepare isolated H-M.[20] These cells can be maintained in culture for up to 10 days without outgrowth of other cell types, and they respond to LPS in a number of different ways, including the selective induction of enzymes.[20] Addition of LPS prepared from Salmonella minnesota Re595 to the cultured macrophages also induces a procoagulant activity (PCA) detectable in cell lysates when assayed in a one-stage clotting assay.[21] The H-M PCA was detected in cells treated with as little as 10 ng/ml of Re595 LPS, and evidence of increased activity was apparent as early as 3 hr after LPS addition, with a maximal response occurring between 7 and 12 hr after LPS.

Previous reports have described the association of tissue thromboplastin with LPS-treated macrophages.[22] The PCA found in LPS-treated H-M has a number of biochemical properties that clearly distinguish it from thromboplastin. These properties are summarized in Table 3. Of interest, however, is the observation that combination of the H-M PCA with tissue thromboplastin results in a 10-fold greater activity than expected from the separate activity of the H-M PCA and the tissue thromboplastin. These data are shown in Table 4 and suggest, together with the biochemical characteristics of the H-M PCA, that the H-M PCA is an enzyme with properties similar to those of activated coagulation

TABLE 3. PROPERTIES OF THE HEPATIC MACROPHAGE PROCOAGULANT ACTIVITY (H-Mo PCA)

H-M PCA	Tissue Thromboplastin
Heat labile 56°C, 30 min	Heat stabile, 56°C, 30 min
DFP sensitive	DFP resistant
DASA sensitive[a]	?
Corrects Factor VII deficient plasma	Does not correct Factor VII deficient plasma
Activates Factor X to Xa	Activates Factor VII

[a] H-M were pretreated for 15 min at 37° with 5 uM diazonium salt of sulfanilic acid (DASA).

TABLE 4. EFFECT ON COMBINING H-Mo LYSATES AND BRAIN THROMBOPLASTIN ON THE TOTAL PCA

	Untreated	Heat Treated (56°C) 20 min	40 min
H-M Lysate[a]	10,000 (100)[b]	3,200 (32)	800 (8)
Tissue Factor[c]	9,800 (98)	9,600 (95)	8,800 (88)
Combination (1:1)	96,000 (960)	34,000 (340)	9,500 (95)

[a] H-M lysates (approximately 5 x 10^5 cells/m^{10} from cultures pretreated for 24 hr with 1 ug LPS/ml medium)

[b] Numbers are representative of a typical experiment and represent PCA in milliunits with neat thromboplastin standard equal to 100,000 milliunits. The numbers in parentheses are percent of activity present relative to the activity of the lysate PCA in NRP being 100 percent.

[c] Rabbit brain thromboplastin standard (1:10)

Factor VII. In this regard Curtiss et al.[23] have recently reported the production of a PCA by LPS-treated human peripheral blood mononuclear cells, which was neutralized by antibody to human Factor VII. This PCA appeared to be membrane bound, could be dissociated by EDTA treatment, and activates purified

Factor X to Factor Xa. Thus, this LPS-induced human moncyte PCA appears to be similar to that first identified in the rabbit H-M. This suggests that the LPS-treated rabbit H-M may express Factor VII or VIIIa on the plasma membrane.

Chapman et al.[24] have also recently described the induction of a PCA associated with murine peritoneal exudate macrophages (PEM), which acts by activating Factor X. Thus, different sources of M can produce a PCA in response to LPS, which is most likely Factor VII or VIIa. This membrane-bound PCA, together with tissue thromboplastin, may produce the initiating signal for localized fibrin deposition and DIC observed after LPS injection.

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DISCUSSION PERIOD WITH DR. ULEVITCH

UNKNOWN: What therapeutic implications do you see in regards to the patient in septic shock?

DR. ULEVITCH: That is a very difficult question. I think that right now our goal is to take the information from these *in vitro* studies and try to develop ways to intervene *in vivo*. For example, one can assume that there is a macrophage factor that suppresses steroidogenesis, which may play a role in sensitizing a patient or an experimental animal to endotoxin. If that factor could be obtained in pure form, you could devise a number of ways to intervene. But at the present time, I think there are not any clear methods.

What I hope to do is to stimulate people who are working with patients or other animal models to try to use this information in their studies and see if it is relevant.

DR. WUSTROW: I have two questions. First, have you tried to use LPS-resistant animals, like the C3H mice, which do not respond to LPS? Second, a factor has been described called tumor necrotic factor. Is that factor similar to what you have shown?

DR. ULEVITCH: There is at least one (and possibly two) strain of mice that is essentially resistant to the toxic effects of lipopolysaccharide. One of the strains is a C3H/HeJ. We have used macrophages from those mice, and they do not produce this steroidogenesis suppressive factor in response to LPS. They do, however, produce it in response to *Listeria*. So that is consistent.

We have also shown that this acute-phase protein that somehow modulates, or interacts with LPS in serum, cannot be induced in the Hej mouse by LPS, but can be induced by other non-specific inflammatory stimuli. Everything we see fits with what is known about that mouse strain and its unresponsiveness.

In terms of tumor necrotic factor, we don't know if it is related or not. There is a whole series of activities now being identified as elaborated from macrophages. In addition to what I have described, there is a factor that has actually been purified now from LPS-stimulated macrophages, which can produce the insulin resistance seen in septic patients or in experimental models of endotoxic shock. It also acts on the fat cell by inhibiting the synthesis of enzymes that convert glucose to lipids. What the relationships are between all of these factors is as yet undetermined; however, they may be critical in mediating injury.

DR. NETA: In your studies on steroidogenesis suppressive factor from macrophages, did you also have a chance to look at the properties of your lymphokine preparation in the similar system?

DR. ULEVITCH: Lymphokines alone are not effective. The lymphokine preparation we have is a crude supernatant, although it is from a T-cell hybridoma. It is very rich in the lymphokine known as macrophage-activating factor, or MAF, but we are not prepared to say which lymphokine is involved. I think everybody who works in this field is faced with the problem of having lots of activity and nothing they can get their hands on in terms of pure molecules.

DR. NETA: I agree, but did you test Rob Schriber's purified interferon/MAF preparations?

DR. ULEVITCH: It is likely that the MAF is, in fact, gamma interferon, and we have not tested any pure preparations of gamma interferon.

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CANINE HEMATOPOIESIS IN A MODEL OF COMBINED INJURY

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INTRODUCTION

The aftermath of a nuclear accident or disaster will present another set of problems to the attending physicians and other medical personnel. They will be presented with alternatives not previously encountered in conventional warfare or in our urban trauma centers. The source of these alternatives or options is the combined-injury patient, that is, the physically injured or traumatized person who has also been irradiated. Each injury can be a serious problem under disaster conditions, but when combined, the single sublethal injury can be quickly transformed into a lethal one. The significance of the combined injury (CI) following a nuclear detonation is evident from the medical records of the casualties treated in Japan. Over 40% of the casualties from Hiroshima and Nagasaki suffered multiple injuries. Experimental studies conducted on various animal models have revealed that the combined effects of injury from more than one energy form or trauma are synergistic in effect. Brooks et al.[1] showed that in a combined insult of thermal burns following total-body irradiation, a dose of 25 r significantly increased lethality following a burn of 20% body area. Alpen and Sheline,[2] in a similar study using a rat model, showed that total-body X irradiation to a sublethal dose of 100 R in combination with an LD50 level of burn injury increased the lethality to 65%. Schildt and Thoren[3] have summarized the characteristics of the combined injury syndrome. It must be mentioned that in their case, the CI is defined as "a complex injury caused by a simultaneous exposure to two (or more) forms of energy or traumata of various kinds." Here it is not required for irradiation to be one of the traumas. Their characteristics include the synergistic effect, a moderation of reaction capacity, impaired wound healing, increased tendency toward shock, and increased susceptibility to complicating infections.

In the context of this presentation, exposure to a sublethal dose of ionizing radiation is considered as the initial cause of the CI, followed by a second stressor either immediately or within several days postexposure. A substantial sublethal exposure to gamma or mixed neutron-gamma radiation severely damages the hemopoietic system of the mammalian species. Predisposition to bacterial sepsis by opportunistic pathogens and impaired wound healing are two of the major consequences of radiation-induced hemopoietic and immune suppression. Functional cells are decreased within days to critically low levels, and precursor and stem cells that are responsible for the regeneration of mature cells may be depressed for weeks after sublethal doses of irradiation.

The development of a large animal model for CI within the context of a nuclear disaster required that we describe, experimentally, the essential features of the radiobiology of acute effects in the canine. The large-animal model is also appropriate for assessing the immunologic, pharmacologic, and surgical modes of intervention following CI. The canine model of CI at the AFRRI has stressed three developmental aspects: (a) establishing the radiobiology of the canine hemopoietic system, (b) choosing a relevant model for peritoneal sepsis, and (c) identifying several choices for physical trauma. This paper stresses the relevance of the first aspect, the radiation-induced suppression and recovery of the hemopoietic system.

MATERIALS AND METHODS

ANIMALS. Healthy, pure-bred, male and female beagles (9-12 kg) were used in these studies. The dogs were treated to eliminate parasitic infections and were immunized against distemper, hepatitis, and rabies. They were observed for 2 weeks before they entered the experimental protocol. They were housed in temperature controlled rooms, in individual stainless-steel cages, and fed kibbled laboratory dog food, which was supplemented once a week with high-protein, canned-meat ration. Water was provided ad libitum.

HEMATOLOGICAL VALUES AND HEMOPOIETIC CULTURE TECHNIQUES.

Peripheral blood was withdrawn from the cephalic vein and bone marrow (BM) was aspirated from the ribs and iliac crest of anesthesized dogs (Bio-Tal, Parke Davis, A.J. Buck & Son, Baltimore, MD) into heparinized syringes. Peripheral blood leukocytes and platelets were counted using a hemocytometer. Plasma from a 1-ml aliquot of blood was harvested for the assay of colony-stimulating activity (CSA). A 5-ml aliquot of blood was used for separation of mononuclear cells (PBMC) using Lymphocyte Separation Medium (LSM, Bionetics, Kensington, MD) and centrifuged at 1500 rpm (400 x g) for 35 min. The mononuclear cell (MNC) layer was harvested and counted for viable nucleated cells in a hemocytometer. BM-derived mononuclear cells were harvested in a similar manner. These cell populations were then assayed for specific hemopoietic progenitor cells.

Granulocyte-macrophage (GM-CFC) and macrophage (M-CFC) colony-forming cells were assayed using the double-layer agar technique as previously described.[4] Briefly, CSA was provided by using pooled plasma from dogs previously injected with Escherichia coli (055:B5) lipopolysaccharide (List Biologicals, Campbell, CA), which was added to the bottom agar layer (7% vol/vol) of triplicate culture dishes. BM-derived MNC and PBMNC were plated in the upper agar layer in concentrations depending on previous treatment. Colonies (> 50 cells) counted after 10 days and 27 days of culture were considered to be derived from GM-CFC and M-CFC, respectively.

Erythroid progenitor cells (CFU-e) were assayed, using the plasma clot technique. For each cell sample, 2 ml of the plasma clot suspension was prepared to contain 0.6 ml heat-inactivated fetal bovine serum, 0.2 ml of 25% beef embryo extract, 0.2 ml of 10% bovine serum albumin, 0.2 ml (0.04 mg) L-asparagine, 0.2 ml of 10^{-3} M 2-mercaptoethanol, 0.2 ml (1 unit) sheep erythropoietin (Ep) (Step III, Cor aught Medical Research Labs., Swiftwater, PA), 0.2 ml cells (concentrations yielding 1 x 10^5 to 5 x 10^6 cells per clot), and 0.2 ml of 37°C bovine citrated plasma. All ingredients were either reconstituted or diluted with supplemented alpha medium (SAM),[35] and control plasma clots contained SAM in place of Ep. Immediately, 0.5 ml of this mixture was

pipetted into each of three 17-mm flat-bottomed wells in Linbro tissue culture plates, allowed to clot, and incubated for 72 hours at 37°C in a humidified atmosphere containing 5% CO_2 in air. Plasma clots were then harvested, fixed with 5% gluteraldehyde, stained with benzidine and giemsa, and scored at 25 X using a conventional light microscope.

PARAMETERS OF COBALT-60 AND MIXED NEUTRON-GAMMA IRRADIATION.

The canines were secured in Plexiglas holders for both types of exposure. Cobalt-60 irradiation was bilateral at a dose rate of 0.1 Gy per minute to various total-body, midline tissue-absorbed doses (MTD). Mixed neutron-gamma irradiation was achieved in a gadolinium-lined exposure room from the AFRRI TRIGA Mark-F pool-type thermal research reactor, operated in the steady-state mode. Bilateral exposure was achieved by a 180-degree rotation at midtime of the exposure. The physical parameters of the free-in-air exposure were an average neutron energy of 0.8 MeV and an average gamma energy of 0.9 MeV, a neutron-to-gamma ratio of approximately 6 to 1, and a dose rate of 0.6 Gy per min. The neutron-to-gamma ratio was achieved by imposing a 15-cm-thick lead wall in front of the reactor core tank wall in the exposure room.

TABLE 1. DEPTH DOSE MEASUREMENTS IN A BEAGLE PHANTOM AND SEVEN BEAGLE CADAVERS

Comparison of Midline to Free-in-Air Doses	Measured in Phantom	Measured in Cadavers (± 1 sigma)
TRIGA Reactor		
Midline neutron dose (% air)	28%	34 (12)%
Midline gamma dose (% air)	210%	206 (21)%
Midline total dose (% air)	49%	52 (11)%
Midline neutron-gamma dose ratio	1.1	1.4 (0.5)
Cobalt-60		
Midline gammma dose (% air)	90%	----

Measurements of dose depth were made at the center of a cylindrical phantom (Table 1). The 15.2-cm diameter of the phantom was approximate to the mean 16-cm diameter determined from the measurement of 54 dog cadavers. The phantom was made of 0.32 cm lucite and filled with muscle equivalent liquid. For mixed neutron-gamma irradiation, the total neutron-plus-gamma dose measured at phantom midline was 49% of that measured free in air; this figure was used to calculate MTD for all dog irradiations. Dose measurements were performed with paired 0.5-cc ion chambers, specifically an A-150 plastic tissue-equivalent chamber with a methane-based tissue-equivalent gas and magnesium chamber with argon gas. Actual animal irradiations were monitored with ionization chambers and sulfur activation foils mounted at fixed positions in the exposure room, to provide corrections for variations in reactor output.

ANTIBIOTIC, FLUID, AND PLATELET THERAPY.

The antibiotics ampicillin, 500 mg, (Polycillin-N, Bristol Laboratories, Syracuse, NY) and gentamycin suflate, 30 mg (Garamycin, Scherring Pharmaceutical Corporation, Kenilworth, NJ) were administered daily until the WBC level reached 1,000/mm^3. Fluid support (lactated Ringer's solution), was administered intravenously as dictated by clinical symptoms. Platelets (3-5 x 10^{10}) obtained by plateletpheresis of donor animals were irradiated with 5,000 rads (cobalt-60 source) and transfused to canines on days 12, 15, and 18 post-irradiation.

RESULTS

LETHALITY OVER A 30-DAY PERIOD (LD50/30) FOLLOWING EXPOSURE TO MIXED NEUTRON-GAMMA IRRADIATION.

Shown in Table 2 are the 30-day mortality values for beagles bilaterally exposed in the AFRRI TRIGA reactor to a range of doses of mixed neutron-gamma radiation. These data placed the LD50(30) value at approximately 1.15 Gy, midline tissue dose. We have not yet been able to accumulate mortality data for cobalt-60 exposure. However, literature values [5-8] place the LD50/30 for

cobalt-60 bilateral exposure at approximately 2.60 Gy. Thus, based on MTD values the RBE for hemopoietic lethality is approximately 2.26.

TABLE 2. THIRTY-DAY MORTALITY IN DOGS BILATERALLY EXPOSED TO RADIATION[A] AT AVERAGE NEUTRON ENERGY OF 0.8 MEV

Dose (Gy)[b]	Dead/Total	Time of Death (Days)[e]
0.75[c]	0/5	No Death
1.00[c]	0/5	No Death
1.25[c]	4/6	20.0
1.25[d]	0/6	No Death
1.50[c]	5/6	10.2
1.50[d]	4/6	11.0
1.75[d]	4/5	19.0
2.00[d]	3/4	6.5
2.25[d]	4/4	8.0
2.50[c]	4/4	4.0
2.50[d]	2/2	6.0

[a]See Materials and Methods

[b]Midline Tissue Dose. Median Lethal Dose taken to be 1.15 Gy. $LD_{50(30)}$ for cobalt-60 exposure taken to be 2.60 Gy (literature values 5-8)

[c]No support

[d]Support in form of fluids, antibiotics and platelets

[e]Mean time to death for all lethalities

The MTD chosen for a sublethal exposure to cobalt-60 gamma radiation in our experimental protocol for the Combined Injury Program is 1.50 Gy. However, an equivalent MTD of mixed neutron-gamma radiation resulted in an approximate LD85/30. This dose had to be reduced to 1.00 Gy before no lethality was observed. Based on an apparent RBE greater than 2.0 using MTD values, the hemopoietic analysis following exposure to mixed neutron-gamma radiation was conducted at an MTD of 0.75 Gy.

PERIPHERAL BLOOD LEUKOCYTES AND PLATELETS.

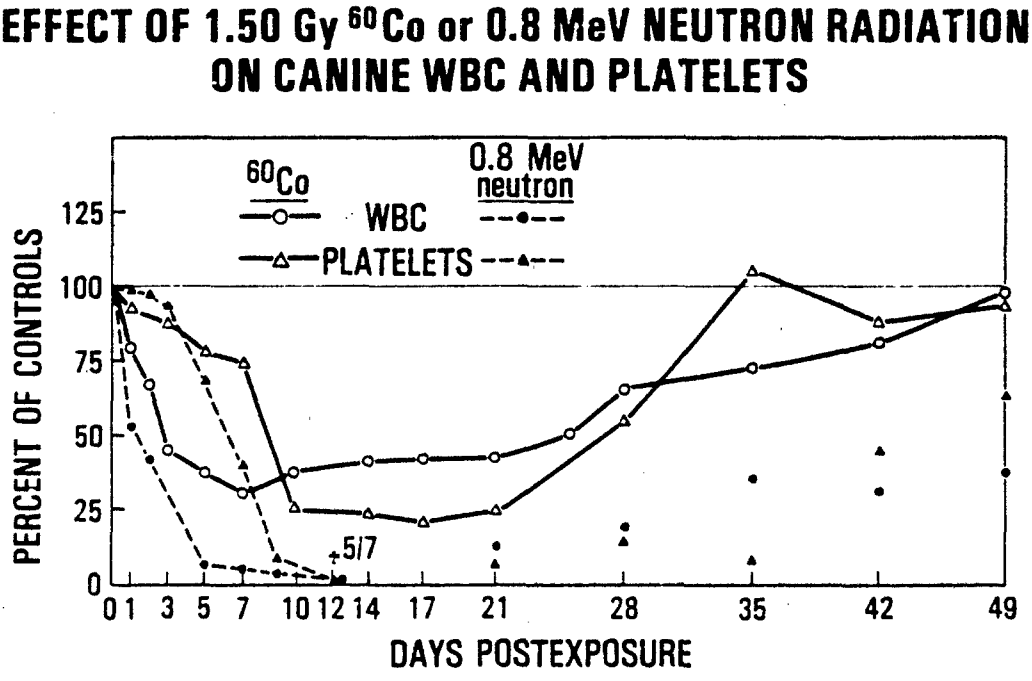


Figure 1. Peripheral blood leukocyte and platelet values as percent of preirradiation values following exposure to either 1.50 Gy gamma or 1.50 Gy mixed neutron-gamma radiation. Mean values 12 dogs (gamma) and 5 dogs (neutron-gamma).

Shown in Figure 1 are the effects of 1.50 Gy cobalt-60 or mixed neutron-gamma radiation on peripheral blood leukocytes and platelets. The 1.50-Gy cobalt-60 gamma radiation significantly reduced circulating levels of white blood cells to a nadir of approximately 30% of preirradiation levels within 7 days postexposure. Platelet levels decreased much slower over the first week and then dropped precipitously to a nadir of 25% of preirradiation levels by day 10. Both parameters (total white cells and platelets) remained depressed through 21 days before recovery toward control levels was observed. Full recovery to preirradiation levels may require 5 to 6 weeks. Also shown in Figure 1 is the response of these circulating elements to 1.50 Gy of neutron-gamma radiation. It is obvious why this dose level has an LD of 85/30: both components decrease at a greater rate to values less than 10% of normal within 5 and 9 days, respectively, for white cells and platelets, and both continue to decrease until death. The dogs that do survive have shown slow recovery of these parameters, with circulating levels at only 50% of normal by 7 weeks postexposure. It is obvious from the standpoint of equivalent MTD's, that equivalent hematological effects are not observed.

SENSITIVITY OF GRANULOPOIETIC (GM-CFC) AND MACROPHAGE (M-CFC) PROGENITORS TO COBALT-60) AND NEUTRON-GAMMA IRRADIATION.

The survival of GM-CFC and M-CFC to both types of radiation exposure over the dose range of 0.25-3.50 Gy is shown in Figures 2 and 3. Calculation of the dose response over the exponential portion of the survival curves (semi-log plot) yields the D_0 value. This D_0 value is a measure of the relative radiosensitivities of (a) different cell types to radiation of the same quality or (b) the same cell type to radiation of different qualities. This allows a measure of the relative biologic effect of exposure to radiation of different qualities seen between cobalt-60 gamma and TRIGA mixed neutron-gamma radiation. The D_0 is that dose needed to reduce a cell population to the 37% survival level on the exponential portion of the survival curve. D_0 is also a measure of the slope of that particular curve in the exponential region where survival is linearly related to the log function of dose.

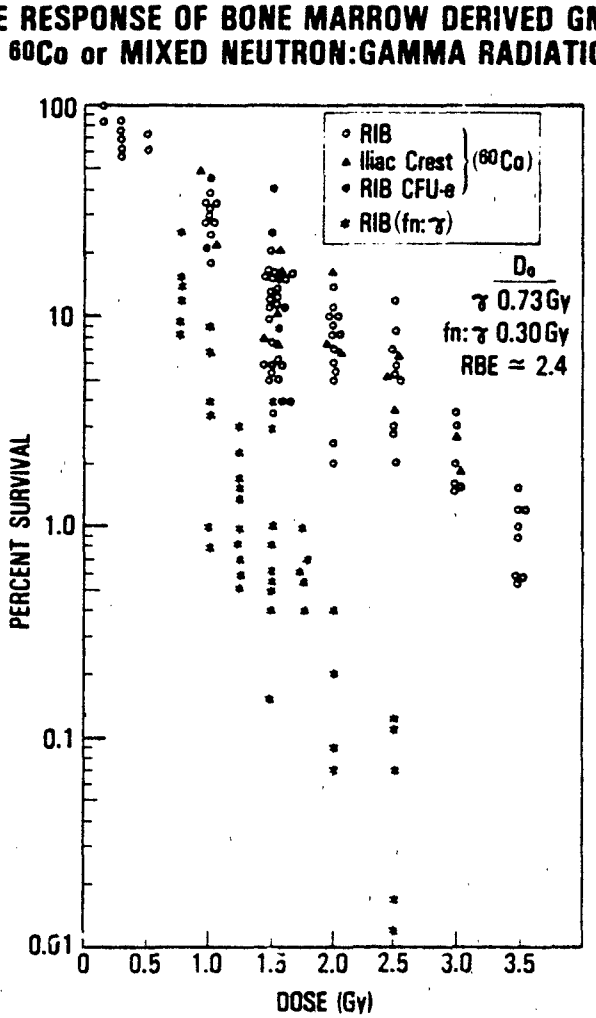


Figure 2. Percent survival of GM-CFC assayed 24 hours post-exposure to cobalt-60 gamma or mixed neutron:gamma radiation over the dose (MTD) range of 0.25 Gy to 3.50 Gy.

The respective D_0 values for GM-CFC harvested 24 hours after exposure were approximately 0.73 Gy and 0.30 Gy for cobalt-60 and neutron-gamma radiation, respectively (Figure 2). The calculation of D_0 values for M-CFC yields a similar response: 0.89 Gy and 0.40 Gy for cobalt-60 and mixed neutron-gamma radiation, respectively (Figure 3). These hemopoietic responses were again calculated from midline tissue absorbed doses. Based on these D_0

values, an apparent RBE of greater than 2.0 exists for sensitivity of GM-CFC and M-CFC to mixed neutron-gamma exposure relative to cobalt-60 gamma exposure. Note that the same total MTD of 1.50 Gy of cobalt-60 or neutron-gamma reduced the GM-CFC survival levels to approximately 12% and 0.9%, respectively.

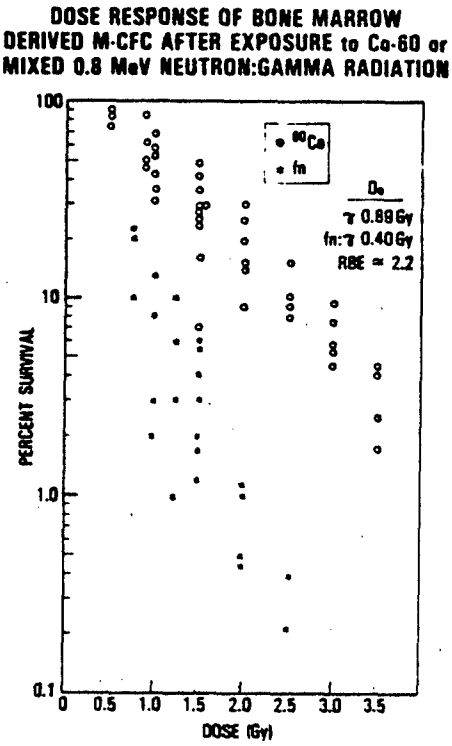


Figure 3. Percent survival of M-CFC assayed 24 hours post-exposure to cobalt-60 gamma or mixed neutron:gamma radiation over the dose (MTD) range of 0.25 Gy to 3.50 Gy.

RECOVERY OF BONE MARROW-DERIVED GM-CFC AFTER WHOLE-BODY EXPOSURE TO 1.50 GY COBALT-60 RADIATION.

Exposure of the canine to 1.50 Gy cobalt-60 gamma radiation reduced the GM-CFC levels to approximately 12% of preirradiation values within 24 hours of exposure (Figure 2). This dose level was sublethal, and recovery of the granulopoietic progenitor cells to pre-irradiation levels required approximately 28 to 35 days (Figure 4). GM-CFC levels remained depressed through 5 days, followed by a marked recovery phase from day 7 through day 21. Then, the recovery rate slowed and gradually reached normal levels over the next 2 weeks, within 35 days postexposure.

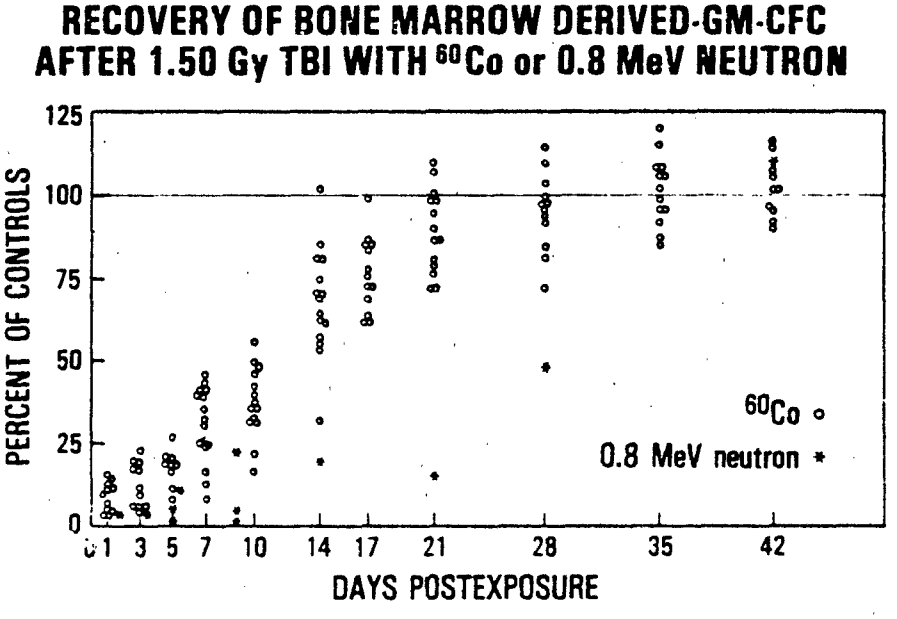


Figure 4. Recovery of bone marrow-derived GM-CFC as percent of pre-irradiation values following exposure to 1.50 Gy gamma radiation. Each value represents individual dog response.

A DIGRESSION: EFFECT OF GLUCAN ON SURVIVAL OF MICE EXPOSED TO A 100% LETHAL DOSE OF COBALT-60 RADIATION.

Glucan is a poly-glycan immunomodulator that is isolated from the inner cell wall of the yeast Saccharomyces cerevisiae, and consists of B 1-3 linked glucose moieties. When glucan was administered to mice 1 day before whole-body lethal irradiation (900 rads, LD100/30 cobalt-60), approximately 50% of the mice survived (>12 months) this otherwise lethal radiation insult (i.e, 100% of controls died by 14 days postirradiation). Experiments are now in progress to examine the radioprotective effects of glucan when administered after irradiation.

It appears that the ability of glucan to enhance survival following irradiation is related to glucan's effects on enhancing hemopoietic recovery. In general, if glucan was administered either 1 day or 1 hour before or 1 hour after sublethal radiation (650 rads cobalt-60), all hemopoietic progenitors (CFU-s, GM-CFC, BFU-e, CFU-e, and HSC) recovered 4-7 days sooner in glucan-treated than in irradiated control mice.[9-11]

DISCUSSION

The groundwork for establishing a combined-injury program is provided by establishing the variables to be controlled and the animal models that must be used to answer specific questions about the expression of combined injuries and the valid extrapolation to human response. In the context of our program at AFRRI, radiation exposure in the sublethal range is a basic component with which a secondary insult of physical trauma is combined. It is imperative, then, that we describe the experimentally essential features of the radiobiology of our canine model. The dose of 1.50 Gy (MTD, cobalt-60 gamma whole-body radiation) in the canine was chosen for several reasons: (a) It is sublethal and yet (b) it decreases the granulocyte-macrophage progenitor cells to a concentration less than 12% of normal, and thus provides a significant hemopoietic stress, (c) STANAG 2083 (NATO Commander's Guide on Radiation Exposure) defines the Radiation Exposure States (RES) where an exposure greater than 1.50 Gy is the highest value a soldier can normally receive without exceeding the emergency risk,[12] and (d) the canine has an approximate

LD50/30 of 2.60 Gy of cobalt-60 radiation (MTD).[5-8] Man has been predicted to have an LD50/30 somewhere between 3.00 and 4.00 Gy, whereas the monkey exhibits an LD50/30 for an average absorbed dose of approximately 5.25 Gy.[13-15] These values place the canine lethality response closer to man; therefore, the canine is an appropriate radiobiological model.

This presentation reports some of the parameters (lethality, hematological value, D_0 value, and hemopoietic recovery) that describe the canine response to radiations of different quality, that is, cobalt-60 gamma and mixed fission neutron-gamma radiation.

LETHALITY AND RBE.

A significant amount of literature establishes the LD50/30 for hemopoietic death of the canine at approximately 2.60 Gy for cobalt-60 gamma irradiation, including X irradiation of the energies 1000 kVp and 2000 kVp [16-20], and an average of 2.28 Gy for exposure to 250 kVp X irradiation.[21-26] These published data indicated a negligible RBE between the cobalt-60 and high-energy X rays but did estimate a small but significant RBE of 0.87 for these higher energy radiations compared to the standard 200-250 kVp X irradation. This value is similar to that reported by Sinclair[27] for LD50/30 values in the mouse, relative to gamma and X irradiation.

Exposure of the beagle to our TRIGA mixed-fission neutron-gamma source resulted in an LD50/30 of 1.15 Gy MTD. This value is significantly lower than the LD50/30 values for fission neutrons of the comparable 1-MeV energy published by Alpen et al.[28] and Ainsworth et al.,[18] with MTDs of 239 rads and 209 rads, respectively. Calculation of the RBE by these investigators resulted in values of approximately 0.90 for Alpen et al. with reference to 250 kVp X irradiation, and 1.38 for Ainsworth et al. with reference to 1 mVp X irradiation. Our observed RBE relative to the standard 250-kVp X-ray exposure would be approximately 2.0, based on the reported average MTD LD50/30 of 228 rads versus our value of 115 rads (an RBE significantly higher than previously reported, as mentioned above). Broerse et al.,[13] however, have

reported a similar RBE for fission neutrons relative to 1-Mvp X irradiation in the primate system. They recorded a total absorbed dose of 2.60 Gy for an LD50/30 from fission neutrons of 1 MeV energy relative to 5.25 Gy for 300-kVp X rays. Thus, the RBE is approximately 2.0 for occurrence of the bone marrow lethality in the rhesus monkey. Similar results have been observed for LD50/30 values in the murine system. Davids,[29] Stewart et al.,[30] and Ainsworth[31] have all reported RBE values of approximately 2.0 for LD50/30 values for exposure to fission spectrum neutrons versus cobalt-60 or X irradiation.

HEMOPOIETIC RESPONSE.

The 1.50-Gy whole-body exposure significantly reduced the circulating blood elements: the white cells, platelets, granulopoietic, macrophage, and erythroid progenitor cells. The nadirs for peripheral blood leukocytes and platelets were observed at 7 days and 10 days, respectively. These levels remained depressed at approximately 25%-35% of preirradiation values through 3 weeks postexposure. Recovery to within normal levels required 5-6 weeks, reflecting the significant although sublethal damage to the marrow stem and progenitor cell pools.

It is this significant and prolonged depression of circulating white cells and platelets that must be considered when selecting medical treatment for the combined-injury patient. The full complement of mature neutrophils, lymphocytes, and platelets will not be available to complete the wound-healing process or resist infections for a critical period of time, depending on dose of radiation and nature of the combined trauma. The medical considerations must be directed not only toward the initial existing condition of the patient but also toward the type of trauma and the situation that will exist 1-3 weeks postexposure.[32]

The reason for the depletion of peripheral blood cells is the sensitivity of their respective progenitor cells and of the pluripotent stem cell populations to the ionizing radiation.[33-36] The sensitivity of the canine GM-CFC and M-CFC, calculated from survival curves over a broad dose range, yield

approximate D_0 values of 0.73 Gy and 0.89 Gy, respectively. The sensitivity of the GM-CFC population measured as number per 10^5 mononuclear cells is consistent with the literature.[33-36] Since we cannot measure the total cellularity of the canine because of experimental design, these values express only the effect of radiation on the relative values of GM-CFC, M-CFC, and CFU-e (per 10^5 MNC) rather than on the absolute number in that marrow location (total per gram of tissue based on total nucleated cells/g of tissue). Wilson et al.,[33] using such a technique for determining the absolute recovery of GM-CFC in weanling beagles, determined the D_0 value to be approximately 70 R following cobalt-60 gamma irradiation. Wilson's results predict that our 1.50-Gy dose reduces the rib marrow cellularity to approximately 60% of normal. Since our D_0 values are similar (73 rads versus 70 R), we could extrapolate our GM-CFC survival fraction to decrease from 12% to roughly 7%, based on total content per gram of rib marrow tissue following 1.50 Gy of gamma radiation.

The RBE based on MTD as observed in our lethality experiments should also be reflected in the radiation sensitivity of marrow progenitor cells, since it is the destruction of these cells that causes the mortality observed over the subsequent 30-day period, known as the hemopoietic syndrome. The calculated RBE's for GM-CFC and M-CFC, as defined by their D_0 values, were indeed greater than 2.0 as observed for the LD50/30. The equivalent biological effect may be viewed from another angle: The respective LD50/30's for gamma (2.60 Gy) and neutron-gamma (1.15 Gy) irradiation should result in a similar percentage survival of marrow progenitor cells. Indeed, the observed value taken from the respective survival curves is approximately 2% survival.

The final aspect of this report is the recovery time necessary for the marrow granulopoietic progenitors to return to preirradiation values. As mentioned above, it is parameters such as this, in addition to the values of the circulating mature cells, that determine the ultimate survival of the patient who encounters a secondary challenge delayed in time from the initial combined-injury or single-radiation insult. Granulopoietic progenitor cells in the canine marrow required 5-6 weeks to reach preirradiation levels. This agrees well with the results of Gerlhartz et al.[34] and Nothdurft and

Fliedner.[35] These collaborators have performed an excellent series of experiments defining the canine hemopoietic response to a range of sublethal doses of low-energy (300 kVp) X irradiation. Our data describing the canine response to 1.50 Gy cobalt-60 and mixed-fission neutron-gamma radiations suggest a significant RBE for the fission neutron irradiation. However, these results are based on midline tissue-absorbed dose. The MTD was established by Bond et al.[37] to be used as a relevant reference dose since free in air, entrance, or exit dose have not been acceptable substitutes. It was their intent to use a value to represent "the dose" received by an animal. This value, MTD, was suggested with full recognition that in irradiating an animal of any size, particularly such as a dog, some degree of inhomogeneity of dose throughout the tissues will exist, no matter what type of radiation is used. In quoting a single value for "dose" received by the animal, it is necessary to fix on the dose received at some fixed location within the animal. Bond states that the ideal is to measure the dose received by a specific critical organ that would correlate with the biological end point, such as the dose received by the bone marrow in the LD50/30 range. This cannot be done, especially with reference to neutron and mixed neutron-gamma radiation. In our particular circumstance, we recognize that a complex dosimetric condition exists (Table 2). Our TRIGA exposure of the canine starts with a 6:1 neutron-gamma ratio free in air with an average neutron energy between 0.8 and 1.0 MeV, and it dissipates to a 1:1 ratio at a midline of unknown neutron energy. We do not know (a) the depth-dose response, (b) the absorbed dose to the critical organ, the bone marrow, (c) the spectral changes (neutron energy) as the dose is absorbed, and (d) the resultant change in neutron-gamma ratio with tissue interaction.

However, two technical reports[38,39] describe the depth dose within canine and pig cadavers and the phantoms from mixed-fission neutron-gamma radiation delivered by a TRIGA Mark-F reactor. Considering the reactor identity, the approximate body sizes of the beagle and miniature pig used (approximately 16 cm wide, 8 cm midline), the similar neutron-gamma fields, and the published depth-dose curves taken as percent of total neutron-gamma surface dose, we

take the liberty of calculating the total dose delivered to the canine at approximately 3-cm depth. The 3-cm depth is based on the proposed location of a large percentage of active bone marrow:

Radiation	Biological Effect	MTD(Gy)	TAR	FIA Dose Surface (Gy)	3cm Depth Dose %FIA	3cm Depth Dose (Gy)
n / γ	1%S GM-CFC	1.50	0.49	3.06	75%	2.30
γ	1%S GM-CFC	3.56	0.90	3.96	95%	3.76
RBE		2.37				1.64

These calculations reduce the RBE from 2.37 based on MTD to 1.64 based on extrapolation using depth-dose data from Wingate[38] and Verreli.[39] The 1.64 value is still considerably higher than the previously reported value of 1.38 by Ainsworth et al.,[18] using the same reactor that Wingate evaluted for depth-dose patterns.

SUMMARY

Combined injuries are caused by two or more forms of trauma. In the context of this paper, the primary form of trauma is the exposure to a sublethal dose of ionizing radiation. The combination of a subsequent sublethal exposure to mechanical or thermal trauma can change two individually sublethal events into a lethal response for the combined-injury host. The establishment of a canine model for radiation-induced aplasia and bacterial sepsis will allow us to investigate the mechanisms involved in mediating the cellular and humoral defenses against sepsis in the irradiated and traumatized host. The large-animal model is also appropriate for assessing the immunologic, pharmacologic and surgical modes of intervention following combined injuries. This paper describes the radiation-induced suppression and recovery of the canine hematopoietic system.

Pure-bred, male and female, young adult beagle canines (weighing 9-12 kg) were used throughout this study. They were bilaterally exposed to either cobalt-60 irradiation (at a dose-rate of 0.1 Gy per minute) or to mixed

neutron-gamma radiation in the AFRRI TRIGA (reactor at a dose-rate of 0.6 Gy per minute) to a predetermined total midline tissue absorbed dose (MTD). The neutron-gamma ratio free in air at the skin surface is approximately 6:1, which dissipates to approximately 1:1 at midline tissue, with an average neutron energy of 0.8 MeV.

The parameters measured were lethality, peripheral hematologic changes, D_0 values for GM-CFC and M-CFC, and recovery of the hemopoietic system following exposure to 1.5 Gy cobalt-60 gamma radiation.

Exposure to 1.5 Gy cobalt-60 radiation was sublethal for 100% of the dogs, and doses of 1.5 Gy and 1.25 Gy in the mixed neutron-gamma field resulted in approximately 85% and 67% lethality, respectively. Exposure to 0.75 Gy was 100% sublethal.

Exposure of dogs over a dose range of 0.50-3.50 Gy of either gamma or mixed neutron-gamma radiation resulted in significantly different D_0 values within the GM-CFC and M-CFC populations. The results show D_0 values for gamma exposure of 0.73 Gy and 0.89 Gy for GM-CFC and M-CFC, respectively. Exposure to mixed neutron-gamma radiation reduced these values to 0.30 Gy and 0.40 Gy, respectively. Calculated RBE values for equivalent biological end-points relative to MTD's for GM-CFC was 2.4 and for M-CFC was 2.2. An approximate biologically equivalent dose for the 1.50-Gy cobalt-60 radiation was taken as 0.75 Gy for neutron-gamma exposure. Hemopoietic recovery in dogs exposed to 1.50 Gy gamma radiation took 4-5 weeks to return to preirradiation levels.

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DISCUSSION PERIOD WITH DR. MacVITTIE

DR. BAUM: I think you ought to clarify your RBE calculation in dogs. Neutrons attenuate rapidly compared to gamma radiation in larger dogs before they get to the midline and thus represent a near uniform exposure. Years ago Drs. Bond and Alpen estimated an RBE of one for the dog neutron to gamma using exposures similar to yours. My question is , do you know the actual dose to the bone marrow before you estimate an RBE of 2.3? You get a lot more neutron radiation to the bone marrow as compared to the midline because of the bone marrow's anatomical location. So probably the RBE is somewhere between one and 2.5. How did you calculate it? Did you use 0.66 of the dose free in air for your midline tissue dose?

DR. MacVITTIE: Yes. However, the tissue to air ratio for our exposures was approximately 0.49 if I am not mistaken.

DR. BAUM: Until you know the actual bone marrow dose it is difficult to determine the true RBE. The midline tissue dose is useful for RBE calculations if restricted to situations in which relatively uniform dose distribution exists. For hematological death, the dose to the bone marrow, if obtainable, would be more appropriate.

DR. MacVITTIE: Your question is very valid and this is one of the areas that is of great concern at AFRRI. We hope to be able to determine the depth dose as as well as neutron energy at various anatomic sites in the near future. That 0.8 MeV value is the average energy of the neutron in air at the surface; the dissipation of it through the tissue is not known yet.

If we use the calculations of depth dose from the TRIGA provided by Verrelli in the pig and Wingate in the dog for a dose at 4 cm depth, we can reduce the RBE to approximately 1.64. Still significantly higher than the value of 1.0 by Bond and Alpen and 1.38 by Ainsworth.

DR. CONKLIN: We have a contract for the development of Monte Carlo computational capability to give us not only dose, but spectral information at any organ site of interest, not just the bone marrow. Hopefully that will be in place within the next 12 months.

UNKNOWN: When you spoke of the dog irradiation, the neutron to gamma ratio was about 6 to 1. Do you expose your dogs unilaterally or bilaterally?

DR. MacVITTIE: Exposures are unilateral, but recipients are rotated at mid-dose so that the other side does get exposed.

DR. McCOY: Have you tried post irradiation treatment with glucan? Studies with interferon show a rapid return of immune competence as related to natural killer cells following lethal cobalt irradiation. Our data suggested there was a significant increase in survival of the animals irradiated and then treated with interferon 24 hours later.

DR. MacVITTIE: To date, most of the data has to do with injecting the glucan prior to exposure, but we have some data post-exposure. Dr. Patchen is in the audience, perhaps she would like to respond to your comment.

DR. PATCHEN: Data that Dr. MacVittie showed were responses to particulate glucan. Particulate glucan administered after irradiation, is not effective, partially because it appears to sensitize animals to endotoxin endogenously released after high doses of radiation. Soluble glucan, which we have just recently obtained, does not sensitize to endotoxin and it looks like it is going to be effective before and after irradiation.

DR. CAMP: Is someone at your shop talking to the operational commanders about the necessity of individual radiation detectors, because right now I think there are only two in the Battalion. It is futile from a medical treatment standpoint to depend on that area dose if you are looking at an individual. The question is, is somebody talking to the big guys up there?

DR. MacVITTIE: Jim, do you want to answer that?

DR. CONKLIN: We go through this every time we make our pitch each year. I think Major Pete Meyers is here from the U.S. Army Nuclear and Chemical Agency and they are well-aware that two dosimeters are issued per platoon. It is a subject of discussion at every NATO meeting that we have. Our allies, I think very correctly, feel everyone should have a dosimeter. That is our feeling. We articulate it every chance we have. It has not been accepted by TRADOC as standard for the U.S. Army, nor for any of the services.

DR. BAINES: In situations where you have either a toxic dose of a chemical or one hit kinetics with irradiation, you may knock out a sensitive population of cells and a resistant population may then repopulate. If an animal has been irradiated one time, and allowed to recover, upon second radiation would you expect to see the same kinetics for recovery as you did in the first case?

DR. MacVITTIE: No, There has been a good deal of information done on "residual damage" to the hemopoietic system. Dr. Baum, has shown that if you exposed rats to 300 rads, of ^{60}Co ionizing radiation and wait a period of time, before exposing them again, residual damage is such that the rat cannot respond nearly as well to the second challenge. The same question is relevant with respect not only to radiation, but as I have tried to indicate, in response to other types of combined injuries.

DR. CONKLIN: I have a question for Dr. Santos. Dr. Santos, in the bone marrow transplant patients at Hopkins who get nominally 1,000 rads whole-body radiation, have you evaluated the T-lymphocyte population as being functional or afunctional post-irradiation?

DR. SANTOS: In autologous or identical twin transplants, we have only done about 30 identical twins, there is little residual host immunity with normal T-cell subsets very early. As they come back what we see is something that is very akin to the recapitulation of ontogeny. Cells possess markers, but are afunctional.

I think the allogeneic system, although similar, is much more complicated because of other things. We have not studied sublethal radiation to see if it, in point of fact, performs similarily.

AD-P003 870

HEMOSTATIC CHANGES IN A CANINE MODEL OF COMBINED INJURY

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INTRODUCTION

Coagulopathies have been reported to occur after many forms of stress and trauma, especially when combined with thermal and radiation injury.[1-16] These thrombohemorrhagic responses are likely consequent to disturbances of the delicate interplay of the hemostatic components (vasculature, platelets, and plasma proteins), resulting in the systemic activation of the clotting-fibrinolytic (and other) systems with eventual depletion of critical hemostatic components. This syndrome of disseminated intravascular coagulation (DIC), an intermediary mechanism of disease, is usually (but not always) associated with well-defined clinical entities. It can manifest as a wide clinical spectrum. For example, if the intravascular clotting process is dominant and secondary fibrino(geno)lysis is minimal, then DIC may be expressed primarily as diffuse thromboses. Alternatively, if the secondary fibrinolysis is dominant, the clinical manifestation will be hemorrhage, by far the most common expression of DIC. Patients often demonstrate combinations of these two clinical manifestations, presenting anywhere in the continuum between diffuse thromboses and/or hemorrhage.[17]

Table 1 lists conditions associated with DIC. In each instance, the systemic response relates to either platelet damage, vessel wall damage, direct plasma protein (clotting factors) activation, or any combination thereof.[17]

The patient with DIC presents a major problem in management. Thus a clear understanding and definition of possible triggering events are desirable and often necessary for efficacious control of the thrombohemorrhagic condition.

TABLE 1. CONDITIONS ASSOCIATED WITH DIC [17]

A. Obstetrical Accidents

1. Amniotic fluid embolism
2. Placental abruption
3. Retained fetus syndrome

B. Intravascular Hemolysis

1. Hemolytic transfusion reaction
2. Multiple transfusions -- Banked whole blood

C. Septicemia

1. Gram-negative (endotoxin)

2. Gram-positive (mucopolysaccharides?)

D. Viremia (Varicella)

E. Solid Malignancy

F. Leukemias

1. Promyelocytic
2. Other

G. Acidosis/Alkalosis

H. Burns

I. Crush Injury and Tissue Necrosis

J. Vascular Disorders

Not all triggers can be avoided or controlled effectively, so therapeutic agents must be used to minimize thrombosis and/or to remove (dissolve) already formed thrombi. The success of that therapy depends on our understanding of the basic mechanisms, pharmacologic and kinetic responses, and availability of appropriate animal study models to assess the efficacy of potentially useful therapeutic agents.

In preparation for the development of a canine model of combined injury, we prospectively evaluated, in vitro, the fluid-phase status of the coagulation system in beagle dogs. Utilizing practical and well-established testing procedures, we obtained baseline data on animals undergoing surgical procedures and/or limited exposure to radiation and/or infection.

MATERIALS AND METHODS

ANIMALS

Sixteen beagle dogs, ranging in weight between 8 and 12 kg, were studied. Of these, two served as controls (temporally housed in the same vivarium), three underwent laparatomy, two underwent bowel resection, two were splenectomized, one was exposed to total-body radiation (cobalt-60 gamma, 150 rad midline total-body), two were irradiated (same as above) and splenectomized within 2 hours, and four were infected with E. coli (10 ml, 2 x 10^8/ml, injected in the colon wall).

SAMPLING

The observation (sampling) period ranged up to 28 days postintervention. Blood was collected by venipuncture into 3.8% Na-citrate (9:1) and centrifuged. The plasma was removed and immediately frozen in aliquots at -70°C. All clotting tests were performed on plasma samples thawed once. For the assessment of fibrinogen/fibrin degradation products (FDP), blood was coagulated with thrombin-EACA, the clot removed, and the defibrinogenated plasma assayed at appropriate dilutions.

TEST PROCEDURES

The following determinations were made: Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), reptilase time (RT), fibrinogen, Factor VIII, Antithrombin-III, and FDP. All clotting tests were performed at 37°C with commercially available reagents (sample dilutions and reagent concentrations accordingly adjusted as described below) and

standardized using a three-dog plasma pool. All clotting times were recorded in seconds (in duplicate) using a fibrometer (BBL Dataclot, Helena Labs., Beaumont, TX).

The prothrombin time[18] and activated partial thromboplastin time[19] were performed essentially as per manufacturer's recommendations (Ortho Diagnostics, Raritan, NJ and General Diagnostics, Morris Plains, NJ, respectively) with the following changes: After reconstitution and immediately before use, the PT reagent was additionally diluted 1:10 in 0.025 M $CaCl_2$, and the APTT reagent 1:32 in tris-chloride ,0.02 M tris (hydroxymethyl) aminomethane and 0.15 M NaCl. buffer, pH 7.2.

The thrombin time was performed by incubating 0.1 ml of sample plasma, 0.1 ml of tris-chloride buffer (described above), and 0.1 ml of bovine thombin (Thrombinar, Armour Pharmaceutical Co., Kankakee, IL), the latter freshly diluted to 3 units/ml in the same tris-chloride buffer containing 0.1 mg/ml bovine serum albumin.

The reptilase time was performed identically to the thrombin time, using the snake venom from Bothrops Atrox (Sigma Chemical Co., St. Louis, MO). The enzyme was diluted to approximate a clotting time equivalent to the thrombin time.

The fibrinogen concentration was determined by a thrombin clottable assay.[20] Basically, this consisted of incubating 0.2 ml of normal dog plasma dilutions (1:5, 1:10, 1:20) with 0.1 ml of bovine thrombin (Thrombinar) at 10 units/ml, and recording the clotting times. All test samples were tested at a dilution of 1:10, and the clotting times were interpolated from the standard curve.

Factor VIII-coagulant concentration was determined by the one-stage procedure[21] using commercially available human Factor VIII-deficient substrate plasma (George King Biomedical, Overland Park, KS). This consisted of incubating 0.1 ml of APTT reagent (Platelin Plus, Ortho Diagnostics), 0.1 ml of Factor VIII-deficient substrate, and 0.1 ml of normal or test plasma at

dilutions of 1:100, 1:200, 1;400, 1:800, and 0.1 ml of 0.025 M $CaCl_2$. A standard curve (clotting time versus log Factor VIII concentration) was used to interpolate values from experimental plasmas, and the values expressed as percent of normal.

Antithrombin-III was determined as heparin cofactor by a two-stage assay, as described .[22] Experimental plamsa samples were tested at a dilution of 1:30, and values were interpolated from the standard curve.

Titration of Fibrinogen-Fibrin Degradation Products (FDP) was performed using the Thrombo-Wellco test (Wellcome Diagnostics, England) as per manufacturer's recommendations.

RESULTS AND DISCUSSION

The normal values for the test procedures described are summarized in Table 2.

TABLE 2. VALUES FOR COAGULATION TESTS ON NORMAL DOG PLASMA

Test	Value (Mean)
Prothrombin time	12 sec
Activated partial thromboplastin time	30 sec
Thrombin time	22 sec
Reptilase time	22 sec
Fibrinogen concentration	250 g/dl
Factor VIII concentration	6.5 units/ml
FDP	Negative
Antithrombin-III	1 unit/ml

It is evident that with the modifications introduced with respect to reagent concentrations and sample dilutions, the parameters described can be reliably quantitated in canine plasma. Figure 1 summarizes the changes observed.

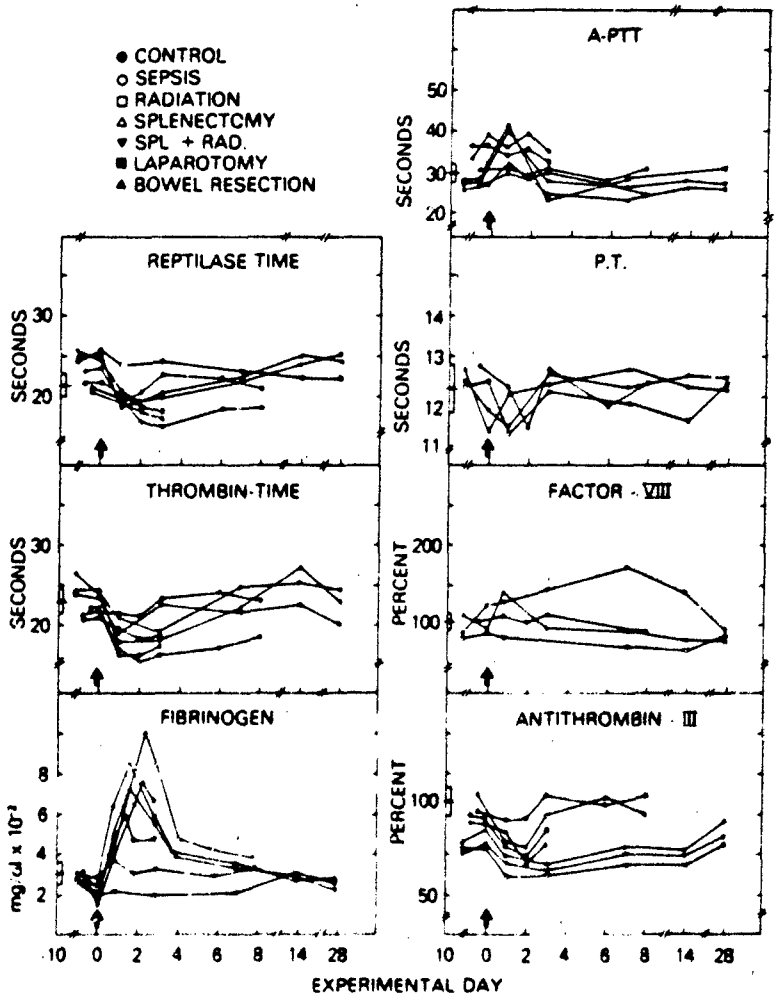


Figure 1. Changes in coagulation parameters in animals (beagle dogs) subjected to experimental procedures indexed on top left of figure. Horizontal axis notes experimental day of blood sampling. Vertical axis notes either clotting time (reptilase time, thrombin time, APTT, PT) or converted concentration value expressed in mg/dl in the case of fibrinogen and in percent of normal in the case of Factor VIII and Antithrombin III. Mean + SD for control animals, observed for a period of 8 days, is indexed on vertical axis. Arrow (day 0) represents time of experimental intervention.

In each instance, the profile of each group is expressed as the mean value of the number of animals studied. Although some animal-to-animal variation was noted, it was observed that control animals studied for a period of 8 to 10 days remained relatively stable with respect to the parameters

addressed. Based on the changes noted in the experimental groups, it appears that global tests such as the PT and APTT are not sensitive enough to note these changes. However, the other tests are, especially Fibrinogen, Factor VIII, and Antithrombin-III.

Radiation alone (150 rads) did not dramatically effect either the intrinsic or the extrinsic coagulation system. Either (a) infection with E. coli as described or (b) controlled surgical intervention, whether in the form of laparotomy, splenectomy (with or without radiation), or bowel resection, resulted in higher concentration of fibrinogen and Factor VIII. The former peaked at about 3 days, and the latter peaked erratically up to 7 days postintervention. This transient phenomenon of acute-phase reactions with respect to Factor VIII and fibrinogen was expected, and has been previously documented.[15,23,24] Concomitant and in accord with these responses were the shortening of the thrombin and reptilase times, indicating a transient hypercoagulable state.

Interestingly, the concentration of the naturally occurring inhibitor Antithrombin-III (heparin cofactor) was slightly reduced, at about 2 days after experimental intervention. It gradually normalized within a few days, depending on the experimental procedure.

The consumption of this important modulator of the coagulation system is generally attributed to the generation of serine proteases systemically,[25] implying that a slight "activation" (generation of active clotting factors) indeed may have occurred.

Soluble degradation products of fibrinogen/fibrin (FDP) were never detected throughout the described experimental procedures, implying that the fibrinolytic response was negligible, if any. All parameters normalized within a period of approximately 10 days.

It is concluded that, with minor modifications, most of the routinely used clotting tests can be reliably applied to assess the fluid-phase status of the coagulation system in the dog. Particularly sensitive to changes were

the tests assessing the concentrations of fibrinogen, Factor VIII, and Antithrombin-III. Results obtained with the described experimental procedures reflect the lack of substantial "activation" of the coagulation-fibrinolytic system. However, these should serve as a basis for further development in establishing an appropriate canine model of combined injury.

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DISCUSSION PERIOD WITH DR. MURANO

DR. WUSTROW: As somebody who talks about the same subject later on, I have some comments and a short question. First of all, I would advise that you should analyze your animals at an earlier time point. Secondly, I am a bit worried that you don't see any changes in the fibrinogen because fibrinogen is an acute phase protein. You should see marked changes. Is your assay system capable of picking up structural alterations?

DR. MURANO: Your first suggestion to analyze at an earlier time is probably a good one. With respect to fibrinogen, I was under the impression that we do see a substantial amount of change with all of the animals except the one which was irradiated but underwent no surgical intervention. Changes that you have documented, I believe, are in rabbits irradiated with 500 rads. I don't know why we did not see a change in the fibrinogen levels in the animal that is irradiated with 1.5 Gy.

I don't think that the spike of fibrinogen that we saw with the other animals has anything to do with the small amount of radiation we used. It has to do with routine, surgical intervention. Fibrinogen does spike, normally, as an acute phase reactant. I suspect, fibronectin will react the same way.

AD-P003 871

IMMUNOLOGIC COMPLICATIONS ASSOCIATED WITH THERMAL INJURY

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INTRODUCTION

Altered cell-mediated immunity following thermal injury has been recognized since the 1950's, when prolonged survival of skin allografts in patients with extensive burns was demonstrated.[1] Further evidence of T cell abnormality was supplied by King et al. in 1963 and confirmed by Rapaport in 1968, in studies showing that the anamnestic response to tetanus toxoid was abrogated following thermal injury.[2,3] A decline in the response of peripheral blood lymphocytes to the T cell mitogens PHA and Monilia has also been reported following thermal injury.[4,5] Direct measurement of the number of peripheral blood lymphocytes by Kaplan and by Neilan et al. has demonstrated a decrease in T cells while B cell numbers apparently remained normal.[5,7] Miller and Claudy found extensive suppressor cell activity in thermally injured patients.[8] Markley measured both cytotoxicity and proliferative T cell responses to mitogens,[9] and found evidence that thermal injury decreases the cytotoxic activity of lymphocytes from spleen, abdominal lymph nodes, and the peripheral blood. He suspected that this effect was caused by a circulating immunosuppressor.

Our own work has shown that burn-patient T lymphocytes, tested during the acute phase of burn care, have a depressed ability to respond in vitro in 75% of the cases studied.[10] The likelihood that lymphocyte response is depressed increases with the severity of the injury. It appears that the observed reduction of patient lymphocyte response is mediated by the generation of nonspecific suppressor T cells.[11] The activity of these cells is usually mitomycin-resistant, and appears to be short-lived since it can be abrogated by a short period of cell culture. We have found that normal lymphocytes display this same pattern of behavior when placed in culture in the presence of serum drawn from patients with thermal injuries. Lymphocyte blastogenic response is significantly reduced, and the generation of suppressor T cells can be demonstrated.[12] While the immunologic literature suggests many possible

serum-borne suppressor candidates, our work and the work of others indicate that the products of arachidonic acid metabolism are major contributors.[12-15]

The importance of an intact T lymphocyte response in protecting the host against infection is difficult to overstate. One of the best indications of the consequences of T cell dysfunction can be seen in immunodeficient patients with DiGeorge syndrome. These patients do not possess any T lymphocyte response; yet, like patients with thermal injuries, they often have essentially normal immunoglobulin levels. These patients are, however, inordinately susceptible to infection with viruses, fungi (e.g., Candida), and Gram-negative organisms of "low virulence" such as enteric bacteria.[16] This same constellation of clinical problems often occurs in patients with severe burns, injuries already shown to result in abnormal T cell function.

Normal T cell function is critical to the burn patient on yet another level, that of the generation of suppressor cells. Such suppressor cells not only function in the regulation of T lymphocytes in the host, but also have at least some degree of influence over almost all of the other immunologically active cells of the body.[17] It is interesting to note that the first observation of inappropriate suppressor cell activity was related to Pseudomonas aeruginosa infection in animals.

Prostaglandins appear to be involved in the generation of many burn injury-related vascular and tissue viability problems.[18,19] A recent report by Heggers et al. described the direct release of large quantitites of prostaglandins from skin cells as a result of thermal injuries.[20] Also, it is now clear that prostaglandins are potent lymphokines[15,21,22] which, along with the leukotrienes, have immunoregulatory properties.[15,23] Exogenous addition of prostaglandins has been shown to prolong allograft survival, suppress in vitro humoral responses to sheep red blood cells,[24] inhibit mitogen stimulation,[25] inhibit mixed lymphocyte reactions,[26] and inhibit cellular cytotoxicity.[27] Finally, it is now clear that prostaglandins are instrumental in the activation of suppressor cells.[15,28]

In the following discussion, I have summarized some of our recent findings relating to the occurrence of depressed lymphocyte response in patients with major thermal injuries, with special emphasis on the participation of the prostaglandins. Evidence that plasma exchange may reverse this deficit in the lymphocyte response is being presented by Glenn Warden.

METHODS

The methods we routinely employ for collecting and storing patient serum samples; for the in vitro study of lymphocyte response; for quantitating endotoxin, interferon, and prostaglandin; and for column chromatography have all been recently outlined in detail.[12,13,29]

RESULTS AND DISCUSSION

During the past 2 years, we have shown that burn-patient lymphocytes, studied during the acute phase of burn care, generally have a depressed ability to respond in vitro. Table 1 summarizes the blastogenic response of lymphocytes from ten example burn patients, when cells were placed in one-way mixed-lymphocyte cultures. All ten patients had third-degree thermal injuries of greater than 50% body surface area. In these studies, each patient served as his/her own control. Sequential serum samples from approximately 60 burn patients have been tested in this manner. As indicated by the Table, profound suppression of lymphocyte response is the general rule in these patients, commencing 1-2 days following injury and continuing until wound closure and metabolic stabilization. Profound suppression was observed whether the patient had sustained a flame burn, scald, or electrical injury, and regardless of sex, age, respiratory injury, or concomitant pathology.

TABLE 1. EFFECT OF BURN ENVIRONMENT UPON LYMPHOCYTE RESPONSE

PATIENT	PATIENT CELLS IN NORMAL SERUM	NORMAL CELLS IN PATIENT SERUM
1	94% sp	95% sp
2	99% sp	99% sp
3	71% sp	72% sp
4	82% sp	78% sp
5	50% sp	45% sp
6	89% sp	82% sp
7	97% sp	98% sp
8	93% sp	97% sp
9	87% sp	89% sp
10	90% sp	90% sp

We have found that normal lymphocytes displayed this same pattern of behavior when placed in culture in the presence of serum drawn at the same time as the cells above, from patients with thermal injuries. Typical suppressive activity of sequential serum samples drawn from patients with major thermal injuries is summarized in Figure 1. We have plotted post-injury day versus the suppressive activity of patient serum as measured in mixed-lymphocyte culture.

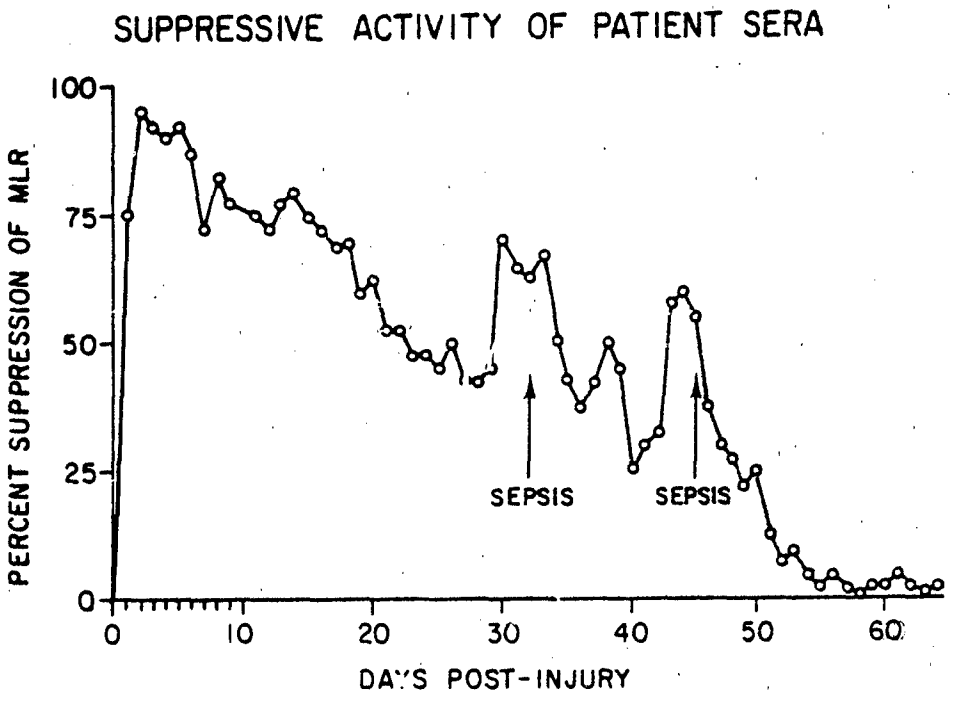


FIGURE 1: Suppressive activity of patient sera.

Such studies reveal the presence of immunosuppressive substances in the serum of almost all patients with major thermal injuries. These substances appear very quickly following injury (they often can be demonstrated within 24 hr) and persist for weeks to months, depending on the severity of the injury. Invariably, patient serum loses its suppressive nature as the wound is surgically closed and the patient approaches hospital discharge. Patients who do not survive have suppressive serum to the very end. In fact, suppressive

activity often reaches a dramatic peak shortly before death. In reviewing these data, we felt that defining the nature of serum-borne immunosuppressive substances would be a great step toward understanding burn-related immunologic changes. We therefore began serum suppressor characterization by Sephadex G-200 column chromatography in a totally sterile, pyrogen-free system.

Figure 2 represents the suppressive profiles of sera drawn from four example burn patients, as determined by G-200 chromatography. We have plotted the serum fractions (their approximate molecular weights) versus their suppressive activity as measured in mixed-lymphocyte cultures. The patients ranged in age from 3 to 37 years; two were scald injuries, and two were flame injuries. All patients had third-degree burns representing approximately 60% of the body

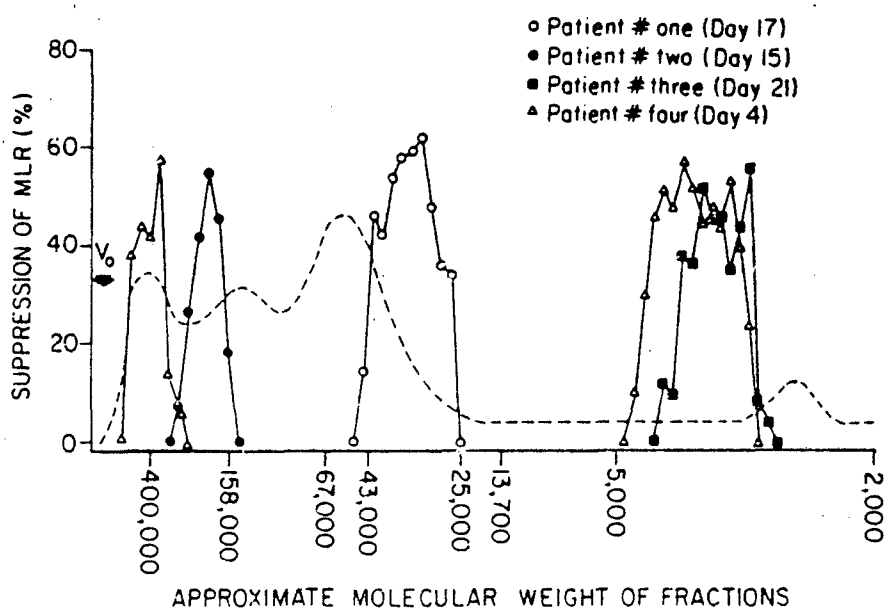


FIGURE 2: Approximate molecular weight of fractions.

surface area. Serum from patient 1 was drawn 17 days post-injury; patient 2, on the 15th day; patient 3, on the 21st day; and patient 4, on the 4th day. Suppressive components of four distinct molecular weight ranges were observed. We have, to date, tested sera from approximately 25 patients in this manner.

These unexpected results indicated that, while suppression was not confined to a single molecular-weight species, suppression was generally confined to six areas. The fact that there was more than one lymphocyte suppressor finally helped us to reconcile earlier mixed results concerning the heat and pH stability, dialyzability, and limulus reactivity of suppressive sera. Conflicting results could now easily be explained on the basis of different suppressors in different test sera.

In order to determine whether or not the occurrence of the six peaks of suppression was due to population selection, we set out to test homogenous sets of serum samples--those obtained from single patients over the acute period of burn care. Results obtained from one of these patients are summarized in Figure 3. The burn patient represented in this Figure sustained an 80%-body-surface-area flame burn (60% was third degree) with a complicating, acute respiratory injury. The sera we tested were drawn at 4 days, 5 days, 30, 47, and 53 days post-injury. When the last sample was drawn, the patient was in septic shock and died the following day. Again, multiple suppressive species were observed in the test sera.

Thus, it was very clear that multiple circulating lymphocyte suppressors existed. While these appeared to be confined to specific molecular-weight regions, they varied in occurrence with different patients as well as within individual patients over time. We were therefore not attempting to define the occurrence and activity of a single suppressive substance, but rather had the task of identifying a whole system of suppressors. The immunologic literature provides a generous supply of possible suppressor candidates. A few of these are listed in Table 2. Our studies to date have considered four of these candidates in some detail.

We have recently reported the presence of interferon in sera drawn from burn patients. The data indicated a correlation between the production of interferon (as measured by antiviral activity of sera in a VSV plaque assay) and the presence of burn wound sepsis. None of the five control sera we tested displayed significant antiviral activity, while one of ten non-specific patients contained approximately 8 units of interferon. Five sera from ten septic patients contained 8-16 units; three had greater than 32 units of interferon. While the interferon levels were certainly not spectacular, they were sufficient to produce some of the immunologic changes observed in the patients.

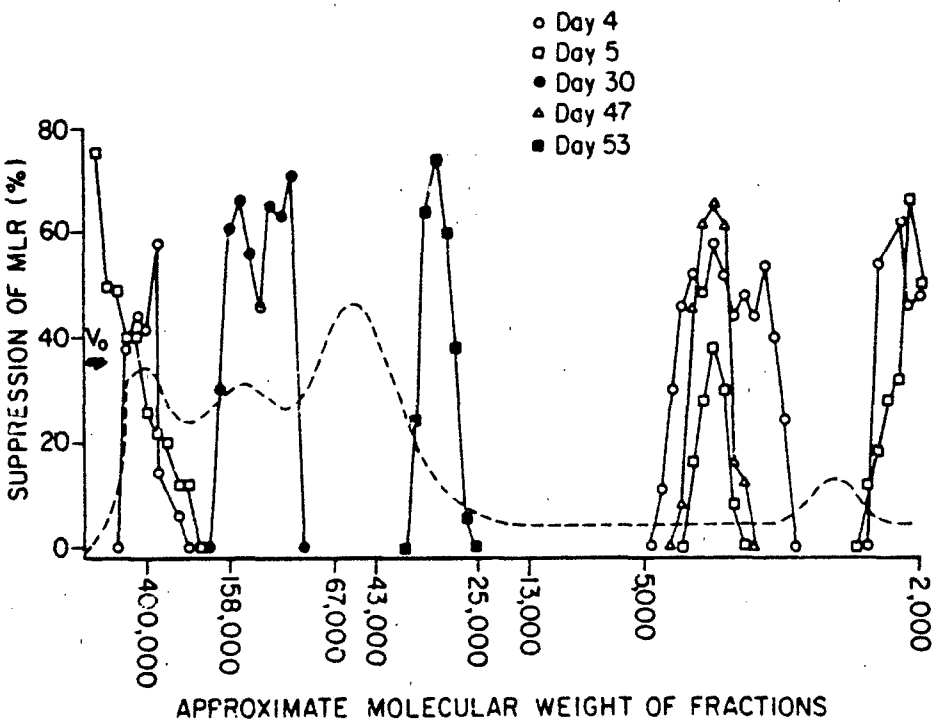


FIGURE 3: Approximate molecular weight of fractions.

We studied the elution pattern of 1000 units of human leukocyte interferon added to 0.5 ml of normal human AB negative serum. Suppressive activity eluted in a region corresponding to approximately 150,000 daltons. Assays of the antiviral activity of the fractions indicated that this suppressive activity was indeed due to the interferon added to the serum. The peaks of suppression eluting at approximately 150,000 daltons from the patient sera represented in Figure 2 and 3 all displayed antiviral activity. We therefore conclude that the 150,000-dalton suppressor peak probably represents serum-borne interferon.

We have shown that endotoxins from a variety of sources and in extremely minute quantities can have a profound effect on lymphocyte response. The very lowest concentration we tested (1.0 ng/well) produced significant suppression using four of the five endotoxins tested. By our calculations, one nanogram

TABLE 2. CAUSES OF T CELL DEFECT IN BURN PATIENTS

Injury itself (burn toxin?)
Circulating mediators (C' activation; AA metabolites)
Nutritional consequences
Hormonal consequences (stress-induced)
Bacterial products
Consequences of medical intervention (iatrogenic):
    Drugs, antibiotics, topicals
    Multiple anesthesia
    Multiple transfusion; blood products
    Fluid resuscitation

of endotoxin is roughly equivalent to the endotoxin contained in 20,000 microorganisms. The presence of endotoxin in serum drawn from patients with thermal injuries can often be confirmed using the limulus ameobocyte lysate assay. With this test, we have been able to demonstrate the presence of endotoxin in approximately 10%-20% of the burn sera we have collected.

When 1.0 ug of endotoxin derived from E. coli 055:B5 was added to 0.5 ml of normal human AB negative serum and subjected to separation on Sephadex G-200, three peaks of suppressive activity could be demonstrated. These corresponded to molecular weights of approximately 600,000, 400,000 and 40,000 daltons. Each of the suppressive peaks proved limulus-positive for the presence of endotoxin. Four of the sera represented in Figures 2 and 3 contained suppressive molecules eluting at molecular weights of 600,000, 400,000, and 40,000 daltons. These were sera drawn from patient 1 (day 17) and from patient 4 (days 4, 5, and 53). Only one whole serum was limulus-positive, was drawn on day 53 from patient 4 during a terminal episode of septic shock. While the other whole sera were not limulus-positive, the fractions collected at the three molecular weights listed above were all limulus-positive, indicating the occult presence of endotoxin in these samples.

We have found that samples of Schoenenberger's human burn toxin are quite suppressive to the mixed-lymphocyte response of normal human cells. Quantities of as little as 100 ng/ml produced statistically significant suppression in our

system. We also found, however, that these burn toxin preparations tested limulus-positive for endotoxin. In addition, burn toxin appeared to have a differential lethal effect when injected into C3H/HeJ (endotoxin resistant) and C3HeB/FeJ (endotoxin susceptible) mice. While susceptible animals displayed an LD_{50} of approximately 30 mg/g body weight, this dose, as well as 50 mg/g body weight, produced no lethality in resistant animals. The presence of endotoxin is therefore suggested.

The complex burn toxin molecule, however, contains at least one more immunologically active compound, which might account for its activity. Our radioimmunoassay procedure revealed that burn toxin also contained large amounts of prostaglandin E. This test revealed prostaglandin concentrations in the neighborhood of 2000 pg/ml burn toxin. The prostaglandin part of this molecule also undoubtedly contributes to its immunologic activity, and may also contribute to the differential lethal effects demonstrated in C3H mice. Therefore, the question of the participation of a discreet "burn toxin" in the immunologic changes following burn injury is currently unresolved.

The role of prostaglandins in the regulation of the immune response has been well established during the past 5 years. Of all the products of arachidonic acid metabolism, PGE_2 appears to have the greatest regulatory significance, and appears to be instrumental in the generation of suppressor T cells. The release of large amounts of prostaglandin (including PGE_2) as a direct consequence of burn injuries had been well documented; however, the participation of prostaglandins in the immunologic changes observed following injury had not yet been determined.

We could readily demonstrate the suppressive activity of prostaglandins (and particularly PGE_2) using our lymphocyte assay system. However, we found that the quantity of PGE demonstrated in patient whole sera by RIA, while generally quite elevated, did not correlate directly with the suppressive activity of the sera in lymphocyte cultures. It is clear from our studies, though, that not all of the PGE present in serum is routinely demonstrated by RIA. For example, we studied the concentrations of PGE detected in serum fractions by RIA after the addition of 1.0 ug PGE to 0.5 ml normal serum. Approximately twice the concentration of PGE was indicated in the 2,000-mw fractions as in the 68,000-mw fractions. Totaling the concentrations of PGE detected in all fractions accounted for only 20% of the PGE_2 added to the column. On the other hand, by following the elution of radioactive PGE_2 from

the column, we confirmed that the greatest amount of PGE_2 eluted with the 68,000-mw (albumin-rich) fraction, with a second peak at mw 3,000. By totaling the radioactivity detected in the fractions, we could account for nearly all of the PGE_2 applied to the column. The inability of the RIA to detect the true amount of PGE, particularly that associated with the albumin-rich fraction, may be due to (a) the affinity of PGE_2 for this carrier, (b) the binding configuration (possible internalization) of the PGE within the albumin molecule, or (c) a technical flaw in our assay procedure. It should be noted that the RIA seriously underestimates the quantity of PGE in the 3,000-mw peak as well. This probably explains the lack of success in previous attempts to correlate patient immunologic depression with the presence and activity of prostaglandins.[30]

The apparent lack of correlation between serum PGE levels and patient immunologic depression can also be explained by the presence of additional suppressive substances in patient sera (Figures 2 and 3). The net effect of many of these substances on lymphocyte response appears to be the same.

Our data support the conclusion that PGE is indeed responsible for the suppressive activity of 3,000-mw fractions from burn patient sera. Suppressive activity of many patient sera correlates very nicely with the amount of PGE detected in the 3,000-mw fraction by RIA. In addition, both the nonspecific procedure of delipidation (the removal of fatty acids, including the prostaglandins) and the addition of monospecific rabbit anti-PGE_2 significantly reduced the suppressive activity of serum fractions.

While the association of prostaglandins (and other fatty acids) with the serum carrier albumin is well documented, we were unable to find any prior descriptions of prostaglandin or fatty acid associating with low-molecular-weight peptides of 3,000 daltons. The literature is replete, however, with descriptions of low-molecular-weight suppressive peptides associated with traumatic injury, advanced cancer, and other medical problems. Many of these previously isolated suppressors may owe their activity to the association of prostaglandin with the molecule.

Finally, it appears that lymphocytes are the source of at least one of the suppressive moieties found in admission serum samples from burn patients. We have arrived at this conclusion by isolating lymphocytes from burn patients.

Lymphocytes were grown in culture dishes, and culture supernatants were harvested after 48 hr. We found that burn-patient lymphocyte (and not normal lymphocyte) supernatants were significantly suppressive not only to MLR, but also to PHA and ConA responses of normal lymphocytes.

It is suggested by these experiments that burn patient lymphocytes do produce a suppressive product(s) (lymphokines) during short-term culture. Radioimmunoassay suggests that PGE is produced in such cultures and, again, may account for at least some of the suppressive activity of cell supernatants.

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DISCUSSION PERIOD WITH DR. NINNEMANN

DR. McCABE: I was a little surprised to see Gram-negative bacilli under your list of organisms in which there was an increased rate of infection. I assume you mean only the intracellular types of Salmonella.

DR. NINNEMAN: We do not consider Salmonella a burn-patient pathogen.

DR. McCABE: I mean, are you implying that it has been demonstrated that in patients with defects in cell-mediated immunity, Gram-negative bacilli infections of the E. coli type, etc., have been demonstrated either in man or experimental animals to be more prevalent?

DR. NINNEMANN: Yes, particularly with intracellular infection.

DR. McCABE: Only intracellular?

DR. NINNEMAN: I understand your implied qualification, and it is well taken. Antibody certainly is important in defense against Gram-negative organisms.

Dr. McCABE: Then that brings up a very germaine point: If for Gram-negative bacilli, host-defense mechanisms are constituted primarily by humoral antibody and phagocytic activity, how do you explain that these are the major types of infections that burn patients have, yet the major abnormality you demonstrated is in T-lymphocyte function, which is active primarily against intracellular parasites? Yet, in the burn patient we don't see problems with pneumocystis, mycobacteria, the characteristic types of organisms that produce infections when there are defects in cell-mediated immunity.

DR. NINNEMAN: That is a very good question, and it is one I don't have a very good answer for. What I can do, however, is cite patients with de George syndrome as an example of a pervading T-cell defect. And the kinds of organism that you see causing problems in de George patients are very similar to the kinds of organisms that you have present in the burn patients, for example fungi, cytomegatovirus, and herpes, as well as Gram-negative infections of low virulence.

But, no, I don't have a good answer for that.

DR. McCOY: I think we ought to keep in mind that the assay used is an MLR. The MLR does require a macrophage, to the best of my understanding.

DR. NINNEMANN: Yes, it does.

DR. McCOY: And the defect itself may be at the macrophage level, not the T-cell level. It is well-known that endotoxin turns off a series of macrophage functions.

DR. NINNEMAN: Yes. And I don't mean to imply that I believe that T-cell function is the only thing that is important. You can't pull out one component of the immune system without considering all of the others, which is essentially what I have done to present this data.

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IMMUNOTHERAPY OF TRAUMA

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INTRODUCTION

Trauma, including burns, is a major cause of death in the United States. Indeed, in the 1-44 age group, it is the leading cause of death. In the individuals who survive the initial insult of burn or major trauma, overwhelming infection is still the major cause of death.[1] It is important to remember that this infectious insult occurs in the presence of a widespread use of antibiotics. Numerous immunologic defects have been demonstrated in burn and trauma patients and in correlative animal models. These defects include alterations in cellular (neutrophil, macrophage and lymphocyte) function[2-4] as well as the presence of putative suppressive factors in serum.[5]

In view of the immunocompromised state of individuals exposed to burns and trauma, a logical approach to successful therapy might consist of immunoprophylaxis using nonspecific stimulators of host defense mechanisms. Several years of studies with immunomodulators in various animals models suggest that such treatment may have potential efficaciousness in burn patients.[6-9] A more recent study in dogs exposed to thermal injury indicated significant increases in survival with animals receiving C. parvum versus saline controls.[10] However, certain side effects of C. parvum may limit its clinical usefulness. Consequently, further studies have been focused on synthetic immunomodulators with fewer side effects.

The attractiveness of immunomodulator therapy for infectious disease in burns is that it abrogates the potential development of antibiotic-resistant strains of bacteria, and in addition, the spectrum of activity of an immunotherapeutant may be considerably greater than that observed using the classical antibiotic therapy. The present studies have focused on a

clinically relevant model of immunosuppression, thermal injury, in examining the therapeutic efficacy of snythetic immunomodulators. The model of immunosuppression was used to optimize the anti-infective activity of the immunotherapeutants.

MATERIALS AND METHODS

Animal Model. Female Hartley-derived guinea pigs, (350-400 g) obtained from Murphy Breeding Laboratories (Plainfield, IN) were used for all experiments. The model for producing the immunosuppression has been previously described.[11] Hypersusceptibility to Pseudomonas aeruginosa 1244 challenge in this model lasts for 7 days with an approximate 100-fold increased susceptibility over control animals. Male BALB/c mice (18-20 g) were used for the CP-46,665 inflammatory cell infiltrate studies.

Drugs. The agents tested were CP-20,961, CP-46,665, muramyl dipeptide (N-acetyl-muramyl-L-analyl-D-isoglutamine), TP-5 (thymopoietin pentapeptide), levamisole, and Li_2CO_3. CP-20,961 and CP-46,665 are synthetic lipoidal amines that were synthesized by Pfizer Inc. (Groton, CT). They have been shown to have distinct immunomodulatory activity,[12] and are administered i.v. in Intralipid. Muramyl dipeptide (MDP) has been shown to enhance immune responses and to increase resistance to infection in a variety of animal models.[13] MDP was purchased from Calbiochem (La Jolla, CA) and administered i.p. in saline solution. TP-5 is the active petapeptide portion of thymopoietin; although known primarily for its thymic differentiation activity, it has many effects on the immune system.[14]

TP-5 was provided by Ortho Pharmaceutical Corporation (Rahway, NJ) to DJS, and was administered i.v. in saline. Levamisole has long been known to have effects on the immune response both in vivo and in vitro.[15] Levamisole was purchased from Sigma Chemical Company (St. Louis, MO), and was administered orally as a sterile solution in 10% dextrose. Lithium has been shown to have a variety of effects on immune functions, both in vivo and in vitro, thought to be mediated via cyclic nucleotides.[16] Lithium carbonate was purchased from Sigma Chemical Company (St. Louis, MO), and was administered orally as a sterile solution in 10% dextrose.

Experimental Design. The use of this animal model allowed us to test immunomodulators in a clinically relevant system. Treatments with drugs were not begun until 24 hours after injury, and did not extend past the time of bacterial challenge, which was 96 hours following thermal injury. Details for each agent tested are given with the results.

Statistical Analysis. Survival data were analyzed at the University of Cincinnati Computer Center using SAS program packages and BMDP life tables and survival functions. For survival curves, two statistical tests were applied, the Generalized Wilcoxon (Breslow) and the Generalized Savage (Mantel-Cox). Cell infiltrate and functional data were analyzed using Student's t test.

TABLE 1. SURVIVAL OF GUINEA PIGS IMMUNE COMPROMISED WITH Li_2CO_3*

	Drug	Placebo
Live	21	20
Dead	19	17
Sum	40	37

* Administered orally (20 mg/kg) in dextrose solution daily for 4 days prior to challenge with 1 LD_{50} of P. aeruginosa.

TABLE 2. MURAMYL DIPEPTIDE* IN IMMUNE COMPROMISED GUINEA PIGS

	Drug	Placebo
Live	11	8
Dead	9	12
Sum	20	20

* Administered i/p. (1 mg/kg) in saline daily for 3 days prior to challenge with 1 LD_{50} of P. aeruginosa.

TABLE 3. LEVAMISOLE* IN IMMUNE COMPROMISED GUINEA PIGS

	Drug	Placebo
Live	4	11
Dead	16	9
Sum	20	20

* Administered orally (20 mg/kg) in dextrose solution 24, 28, and 72 hours prior to challenge with 1 LD_{50} of P. aeruginosa.

TABLE 4. VP-20,961* IN IMMUNE COMPROMISED GUINEA PIGS

	Drug	Placebo
Live	56	58
Dead	64	62
Sum	120	120

* Administered i.v. (10 mg/kg) in Intralipid either 24, 48, or 72 hours prior to challenge with 1 LD_{50} of P. aeruginosa.

RESULTS

As shown in Tables 1-4, neither lithium carbonate, muramyl dipeptide, levamisole, nor CP-20,961 improved the survival of the immune suppressed guinea pigs that had been challenged with P. aeruginosa. Lithium (20 mg/kg, p.o.) was administered beginning 24 hours post-injury and given daily for 4 days. The animals were challenged with 1 LD_{50} of P. aeruginosa 96 hours post-injury. MDP (1 mg/kg, i.p.) was given i.p. at 24 hours post-injury and again at 48 and 72 hours, prior to a challenge with 1 LD_{50} of P. aeruginosa 96 hours post-injury. Levamisole (20 mg/kg, p.o.) was given 24, 48, and 72 hours post-injury, prior to a challenge with 1 LD_{50} of P. aeruginosa 96 hours post-injury. Similarly, CP-20,961 (10 mg/kg, i.v.), given as a single dose either

24, 48, or 72 hours post-injury, had no effect on survival after challenge with P. aeruginosa (Table 4). The results obtained when CP-20,961 was administered at any of these three times were statistically indistinguishable; thus, the data in Table 4 are the compilation of all three test groups.

Another lipoidal amine, CP-46,665, given as a single dose (0.3 mg/kg, i.v.) 24 hours post-injury but prior to challenge with P. aeruginosa 96 hours post-injury, significantly improved survival (Table 5). Other experiments (not shown) demonstrated that this protective effect was still seen when CP-46,665 was given 72 hours post-injury (only 24 hours prior to bacterial challenge).

TABLE 5. CP-46,665* IN IMMUNE COMPROMISED GUINEA PIGS

	Drug	Placebo
Live	34	21
Dead	6	19
Sum	40	40

* Administered i.v. (0.3 mg/kg) in Intralipid once 24 hours after injury but 2 days prior to 1 LD_{50} challenge of P. aeruginosa.

Likewise, the active portion of thymopoietin, TP-5 (0.1 mg/ke, i.v.), beginning 24 hours post-injury and repeated at 48, 72, and 96 hours post-injury prior to challenge with P. aeruginosa at 96 hours post-injury, significantly improved both survival time and mean survival time (Table 6). The proportion surviving was increased from 40% to 80% and MST from 6.9 days to 11.6 days.

TABLE 6. TP-5* IN IMMUNE COMPROMISED GUINEA PIGS

	Drug	Placebo
Live	24	12
Dead	6	18
Sum	30	30

* Given in multiple doses (0.1 mg/kg, i.v.), beginning 24 hours post-injury and repeated every 24 hours up to and including the day of bacterial challenge.

TABLE 7. PMN LATERATIONS FOLLOWING ADMINISTRATION OF CP-46,665* TO IMMUNE COMPROMISED GUINEA PIG

	Hours Post-Drug		
Assay	24	48	72
Cidal Activity[t]	No	No	No
Enzymes	No	2 x MPO	2 x MPO
Chemotaxis[tt]	Normalized	50% restored	50% restored

t Cidal assay: Peripheral blood PMN's incubated with normal serum for 3 hours with Pseudomonas or E. coli at ratio of 5:1 or 50:1 (bacteria:PMN) and then washed and plated.

tt CTX: 10 mg FMLP injected i.p. and 120 minutes later peritoneal cavity lavaged. Number of cells and differential done on washout. Normally a twofold increase is observed.

* CP-46,665 administered i.v. at 10 mg/kg

As demonstrated in Table 7, certain functional characteristics of PMN's were restored following CP-46,665 treatment in the immune suppressed animals. PMN myeloperoxidase was increased twofold above the compromised controls which had not received CP-46,665. Concurrently, the number of PMN's infiltrating the peritoneal cavity following intraperitoneal adminstration of the

chemoattractant FMLP was restored to 50% of that seen in control animals. Additional studies in mice suggested that CP-46,665 was inducing a profound increase in PMN's in the peritoneal cavity in a dose-related manner (Table 8). The response time was rapid, occurring by 4 hours and lasting for 48 hours. Mononuclear cell infiltrate appeared to be minimal.

TABLE 8. EFFECT OF I.P. INJECTION OF CP-46,665 ON PERITONEAL CELL POPULATIONS IN BALB/c MICE

			Drug Dose		
Time Post-Injection	Saline	Intralipid	10 mg/kg	5 mg/kg	0.3 mg/kg
4 Hours:					
Cell No.[t]	2.8 ± 0.3	2.7 ± 0.4	5.9 ± 1.3[tt]	3.4 ± 0.6[tt]	2.5 ± 0.4
% PMN[b]	3.2 ± 1.9	12.6 ± 7.8	71.0 ± 2.6[tt]	44.1 ± 7.5[tt]	14.4 ± 3.3[tt]
24 Hours:					
Cell No.	3.1 ± 0.6	4.2 ± 1.2	15.6 ± 2.1[tt]	14.2 ± 2.2[tt]	4.5 ± 0.5
% PMN	7.8 ± 5.3	18.4 ± 8.5	44.9 ± 3.4[tt]	52.4 ± 8.6[tt]	18.0 ± 4.7
48 Hours:					
Cell No.	3.9 ± 0.2	2.9 ± 0.3	8.0 ± 1.6[tt]	8.1 ± 0.8[tt]	3.2 ± 0.3
% PMN	0.9 ± 0.2	1.0 ± 0.4	14.1 ± 0.4	12.2 ± 2.9[tt]	2.1 ± 0.8
72 Hours:					
Cell No.	3.3 ± 0.4	5.6 ± 1.6	4.5 ± 0.7	6.9 ± 1.1[tt]	2.3 ± 0.3
% PMN	1.1 ± 0.4	5.5 ± 4.3	7.8 ± 3.0	1.1 ± 0.2	1.4 ± 0.4
96 Hours:					
Cell No.	3.8 ± 0.5	8.6 ± 2.8	6.2 ± 0.3[tt]	5.1 ± 0.4	3.0 ± 0.4
% PMN	0.4 ± 0.2	2.5 ± 1.7	0.8 ± 0.2	1.3 ± 0.4	0.9 ± 0.2

[t] cell no./mouse x 10^{-6}
[tt] Mean ± SE
* p 0.05

DISCUSSION

Interest in immunomodulators grew about 10 years ago because of their potential use in the treatment of malignant disease.[17] The earliest agents used were intact microbes or microbial products. These had the disadvantage of undesirable side effects. Moreover, their complex chemistry precluded the definition of a precise pharmacologic mode of action. Consequently, research efforts have led to discovery of a variety of chemically defined compounds having immunomodulatory properties.

Most of these agents also stimulated host resistance to infection. However, clinical use for this purpose has been sporadic.[18] The prevalence of infection as a cause of death in burned individuals suggests that immunomodulators could be of benefit in this group of patients. A previous study in animals immune suppressed by thermal injury showed a beneficial effect of the bacterial immunomodulator Corynebacterium parvum (Coparvax). A clinical study is presently under way. Even though efficacy may be demonstrated, C. parvum has the disadvantages of side effects and the complex pharmacology cited above. Consequently, we have been interested in synthetic compounds.

In this report we describe a clinically relevant small animal model that is useful for evaluating immunomodulators for potential use in burn patients. Using this model, we examined six low-molecular-weight compounds for the ability to enhance resistance to a challenge with P. aeruginosa. Only two, CP-46,665 and TP-5, had any significant beneficial effect. This clearly stresses the fact that agents should be screened for efficacy in clinically appropriate animal models, even though they may have been shown to have anti-infective properties in normal animals. In addition, we believe effective and ineffective compounds can be useful as probes to identify more precisely the sites of immunologic dysfunction that accompany burn injury.

At least three (not mutually exclusive) mechanisms of action for the active immunomodulators in this study may be envisioned. First, the immunomodulator may directly repair an immunologic lesion produced by burn

injury and thereby restore that pathway to full functioning capacity. Second, the compound may promote an alternative route ("detour") around the lesion in an immunologic pathway and thereby restore function. Third, immunomodulators may provoke compensatory activity of immunologic pathways that are relatively unaffected by burn injury.

In this regard, it is important to note that (as demonstrated in Tables 7 and 8) CP-46,665 induced a partial restoration of an *in vivo* chemotactic defect in the immune suppressed guinea pigs and elicited a significant PMN infiltrate in the peritoneal cavity of mice. Therefore, it may be suggested that the *in vivo* protective effect of this agent may be an enhancement of PMN responsiveness at the site of infection in the compromised animal. Further studies are obviously needed to substantiate this suggestion.

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DISCUSSION PERIOD WITH DR. LOOSE

DR. ULEVITCH: I was struck by the large increment of complement activation that you showed. Have you tried simply complement depleting the rats before you put them through this trauma to see if mortality shifts?

DR. LOOSE: Like cobra venom, or immune complexes?

DR. ULEVITCH: Yes.

DR. LOOSE: No, we have not.

DR. SANTOS: There is another form of trauma that can give you increased antibody formation. This is with cytotoxic chemotherapy. Are you aware of that?

DR. LOOSE: Yes, I am.

DR. SANTOS: You may be bypassing the T-helper cell by some of the materials that are released. You have suggested that this was really a change in population. Do you have any evidence for that?

Most of the evidence with the other observations of our own and other people are that, in fact, we are bypassing the requirement for the T-cell helper in antibody formation. This might be important.

Without lymphocyte transfers, what you did show was a serum depressive action. Is it possible that with changed environment you have a transient appearance of T suppressors?

DR. LOOSE: Based on a cell to cell comparison of peritoneal macrophages taken from trauma animals we believe we have a changed population of macrophages which are better processors.

DR. McCABE: Your differences between killing, were 23 percent to 16 percent and 12 percent to 8 percent. Are these significant?

DR. LOOSE: They are statistically significant. The system that we use is a microscopic assay developed to quantitate both phagocytic uptake as well as the intracellular killing. This system has been optimized to give us a measurable defect, in a minimal period of time.

We can expand a time frame and expand killing, but that then raises questions of viability.

DR. McCABE: It must take a tremendous number of observations to make a difference in the killing of 6 percent statistically significant, doesn't it?

DR. LOOSE: Yes.

AD-P003 873

DISTURBANCES IN BLOOD COAGULATION AND THROMBOCYTE FUNCTIONS AFTER COMBINED INJURIES WITH RADIATION AND TRAUMA

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INTRODUCTION

After any radiation accident there exist[1] the difficulty of determining the radiation dosage. Gorizontov[2] used the alteration of the blood coagulation as a means of biological dosimetry. He divided the hemorrhagic symptoms into four stages. In grade I with 20 gray whole-body irradiation, there was a marked prolongation of the clotting time with a thrombocytopenia. In grade II with 35 gray, slight mucus-membrane bleeding was observed. In grade III with 50 gray, bleeding of the retina and an occasional general hemorrhagia exist. In grade IV with more than 60 gray, profuse and sometimes fatal general bleeding occurs.

Radiation injuries might take place not only in the commercial situation with the production and generation of nuclear energy but also after any explosion of nuclear arms. In those instances, most radiation injuries will be combined with burns, fractures, hematomas, or even simple skin wounds. We have to face a new disease[3,4] summarized by the term "combined injuries." Furthermore, combined injuries may also be present as in the treatment of cancer therapy with high doses of radiation. In these circumstances, the disturbances of the coagulation system, potentiated by the radiation and trauma, are of particular interest. In this context when we speak about combined injuries, we are using the classical definition of Wintz (1923): "An injury that occurs only after exposure of the radiated tissue to still another trauma".[5] Radiobiological changes are therefore essential in the pathogenesis of combined injuries.

Boegelein et al.[1] described a marked hypercoagulability in the thrombelastogram and a prolongation of the euglobin lysis time in his experiments with NMRI-mice receiving 3.0 to 3.5 gray whole-body irradiation and a small open skin wound. In our animal experiments with rabbits, we analyzed the disturbances of the plasmatic coagulation system 2 and 24 hours after

sublethal irradiation and the influence of an additional skin wound or hematoma. This enabled us to distinguish between the effect of the hematoma itself and the hematoma or the skin wound both combined with radiation damages. The autologous hematoma served as a known model that leads to morphological and functional alterations in the lung, like the respiratory distress syndrome.[6,7]

Besides the plasma coagulation factors, undisturbed hemostasis is dependent on thrombocytes and their normal functions. Dienstbier et al.[8] observed a primary thrombocyte function disability after whole-body irradiation, and Hohage et al.[9] described a reduced platelet aggregation with collagen after whole-body irradiation of rats with 7.0 gray.

Platelet number is diminished by radiation, a further reduction of the thrombocyte adhesion and aggregation would increase significantly the risk of acute bleeding. Correspondingly, we analyzed changes in thrombocyte functions after sublethal radiation or surgical trauma alone and their combination as a model of combined injuries.

MATERIAL AND METHODS

Animals. Forty-five male and female rabbits (purchased from Thomae, Biberach a. d. R.), ages 3-4 months and a body weight of 2.3-3.1 kg, were used. All animals were kept in single cages and provided with lab chow and water ad libitum.

Radiation. Under the X-ray machine (type M. G. 300, C. H. F., Muller, Hamburg), the animals were irradiated in a defined position at 60 cm with 250 kV and 123 mA in plastic cages without any anesthestic. The filter consisted of 0.77 cm Cu, the self-filtration of the tubing was 6.0 mm Al, and the thickness period of the radiation was 1.9 mm Cu with a dose rate of 0.47 gray/min.

During sham irradiation, the animals were placed in the same defined position beyond the not-working X-ray tube for the same time. The dose rate of the irradiation was measured continuously by a Duplex-dosimeter (Physik.-techn. Werkstatten, Freiburg i. Br.) with a total dose of 5.0 gray.

Anesthesia and Operation. During epontol anesthesia (propanidid 30 mg/kg body weight i.v.) in all animals 2 hours after irradiation, a 0.8-mm catheter was placed through the external jugular vein into the inferior vena cava up to the diaphragm to facilitate easy blood drainage. The animals were divided into five groups (Tab. 1). Two hours following irradiation or sham irradiation, 10 ml blood/kg body weight equivalent to 12.5% of the total circulating blood volume was drawn. Each animal of group (H) and (R + H) received blood immediately as an autologous hematoma periossal and intramuscularly into the right thigh. In group (R + W), a skin patch 2.5 cm in diameter was excised from the left thigh, followed by skin closure with four sutures and bandage under aseptic conditions. For all the animals the epontol anesthesia was continued by several reinjections using a mean of 80 mg/kg body weight intravenously. The animals awoke 3-5 minutes after the last injection. All of the coagulation analyses were done at 2 hours (except both the hematoma groups) and 24 hours after sham or complete irradiation.

Plasma Coagulation. Percent hematocrit was determined using an Autocrit centrifuge (Clay, Adams, USA), total protein content was measured photometrically with the biuret method read from a standard curve in mg/100 ml serum. The activated partial thromboplastin time (APTT) was measured in seconds with Actin™ (activated cephaloplastin reagent, Mertz and Dade Co.) in the coagulometer according to Schmitgen and Gross. Factor X was determined after the method of Aurell et al.[10] with the chromogenic substrate S-2222 (Kabi Co., Munchen) and read from a standard curve in percent. Fibrinogen levels were analyzed photometrically with test reagents of Boehringer Co., Mannheim, at 640 nm and multiplied with a conversion factor according to the hematocrit. The inhibitory activity of the plasma coagulation cascade like antithrombin III (AT-III) and antiplasmin (AP) were measured photometrically with the chromogenic substrates S-2238 and S-2251 (Kabi Co., Munchen) at 405 nm and read in percent from a standard curve.

Thrombelastogram (TEG). According to the method of Hartert et al.,[11] 0.25 ml blood was measured in cuvettes prewarmed at 37°C. We analyzed the reaction time in minutes from the starting point to the onset of the oscillation of 1 mm, the clot-forming time k in minutes from the onset of the oscillation to the point with an amplitude of 20 mm, and the maximum amplitude during 1½ hours in mm.

Thrombocyte Functions. Platelet number was determined in a Coulter Counter (Toa Co., Japan). The platelet adhesion was measured according to Morris et al.[12] by a standardized contact with glass-perls and their retention expressed in percent. The thrombocyte aggregation was analyzed nephelometrically according to Born et al.[13] The thrombocytes were aggregated in platelet-rich citrated plasma with collagen.

Statistics. In all groups, the means, standard deviations, and standard errors were compared, and the significances were calculated with the rank test of Mann-Whitney modified by Wilcoxon[14].

RESULTS

The hematocrit caused by the blood withdrawal decreased significantly in all the groups at 24 hours after irradiation. As an overall test of the plasma coagulation cascade, the activated partial thromboplastin time (APTT) did not change at all in the control group (C) or after 2 hours in the irradiation (R) and combined injury groups (R + W). Twenty-four hours after irradiation with and without additional trauma, however, the APTT increased significantly compared with the 2-hour levels (R and R + W). Furthermore, in all the irradiated groups (R, R + H and R + W) the APTT was elevated significantly after 24 hours compared with the controls (Fig. 1).

Factor X and Fibrinogen increased dramatically in all radiated groups (R, R + H, R + W) after 24 hours compared with the 2-hour data. However, Factor X also increased in the control group. The elevation of fibrinogen levels in the combined injury group (R + W) was significantly greater at 24 hours than after irradiation or hematoma alone (Fig. 2). In contrast, there was no change of the total protein content in all the groups (Fig. 3).

The antithrombin-III activity (AT-III) tested with chromogenic substrates started from a very low level of about 80% at 2 hours. At 24 hours a slight increase (R + W) and a significant increase (R, H, C) were observed in the different groups. In contrast, the antiplasmin activity showed a significant increase at 24 hours after irradiation whether with or without a trauma compared with the 2-hour level or the control level (Fig. 4).

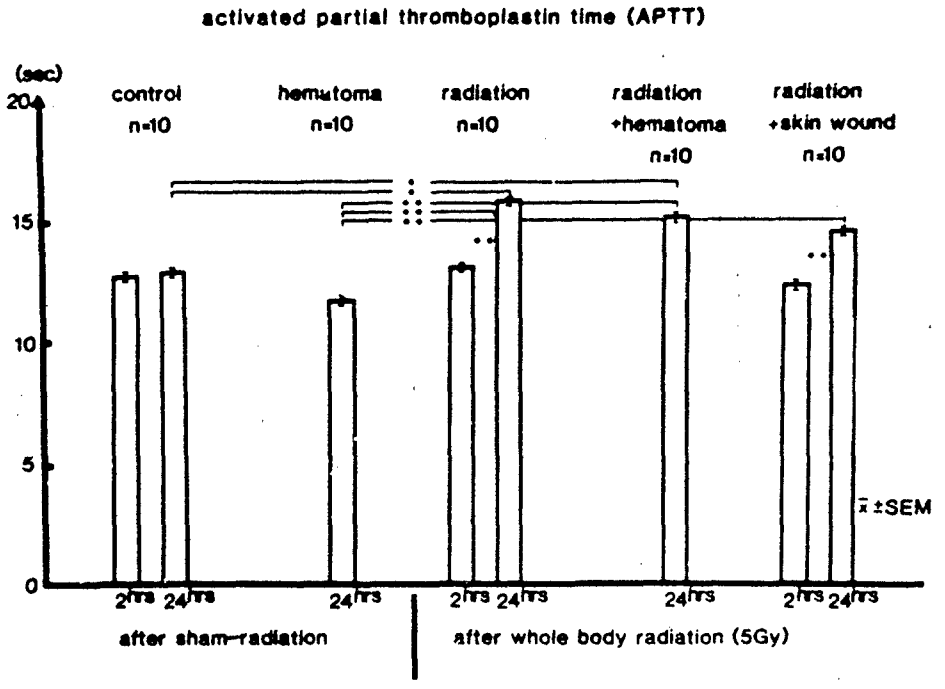


Figure 1. Activated partial thromboplastin time after sham or whole-body irradiation with and without additional trauma. *: $\alpha < 0.05$; **: $\alpha < 0.01$.

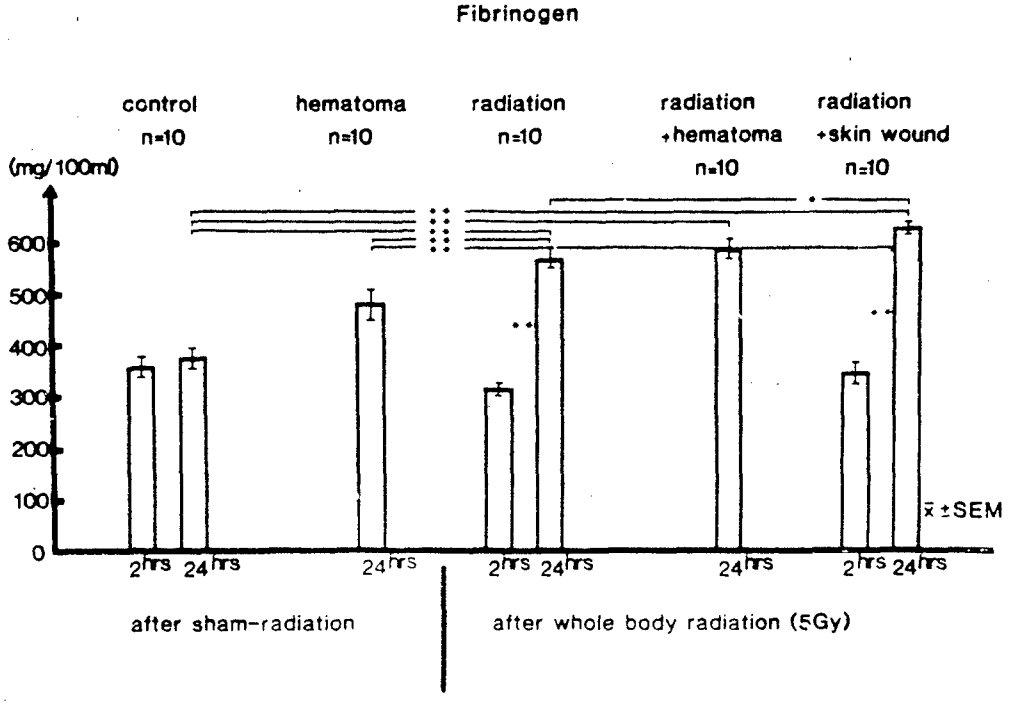


Figure 2. Fibrinogen concentrations after sham or whole-body irradiation with and without additional trauma. *: $\alpha < 0.05$; **: $\alpha < 0.01$.

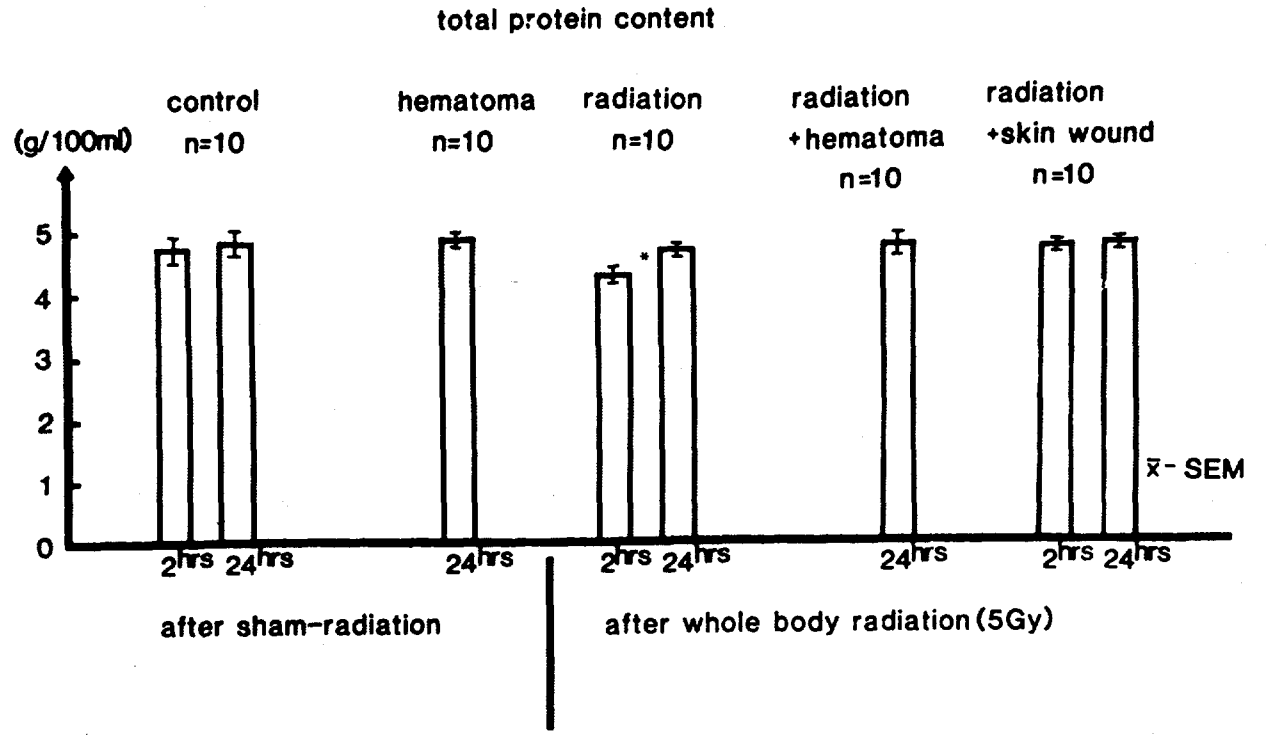


Figure 3. Total protein content in the plasma after sham or whole-body irradiation with and without additional trauma. *: $\alpha < 0.05$.

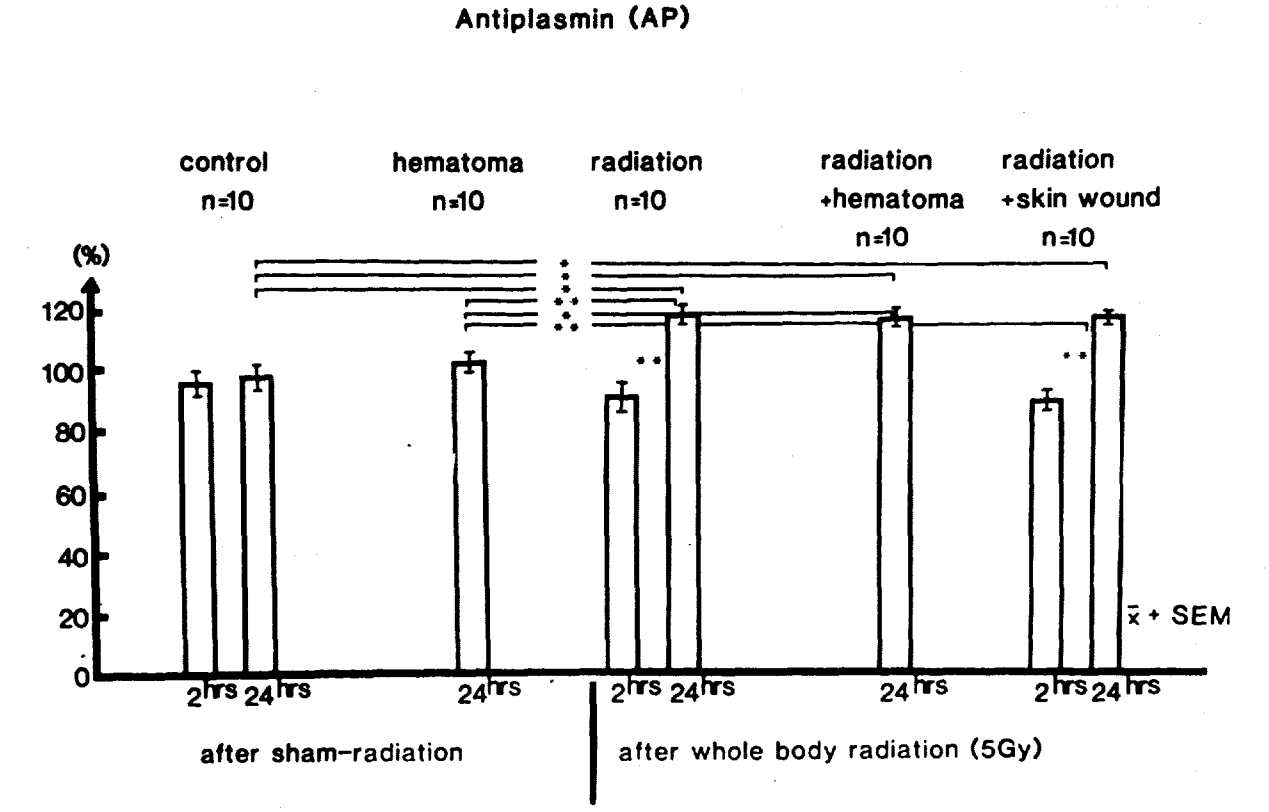


Figure 4. Antiplasma concentration after sham or whole-body irradiation with and without additional trauma. *: $\alpha < 0.05$; ** $\alpha < 0.01$.

In all the groups, the numbers of thrombocytes decrease similarly to about 150,000 mm^3, due to the blood loss (Fig. 5). In the thrombelastogram, the reaction time r (dependent on the platelet count) decreased markedly at 2 hours after radiation (R and R + W) when compared with the controls (Fig. 6). At 24 hours, however, the reaction time r was significantly prolonged in all the radiated groups (R and R + H) and most prominently in the (R + W) group. A similar pattern could be seen from the clot forming in all the radiated groups, with a decrease at 2 hours and a progressive increase at 24 hours time, k. No significant changes of the maximal amplitude could be recorded in all the groups.

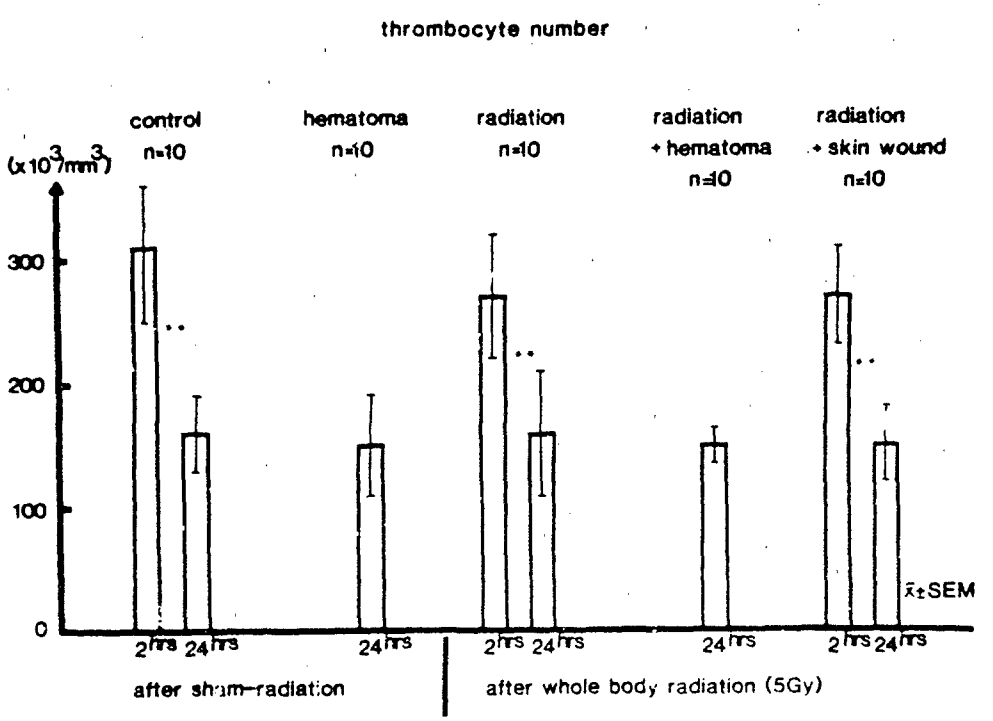


Figure 5. Thrombocyte number after sham or whole-body irradiation with and without additional trauma. **: $\alpha < 0.01$.

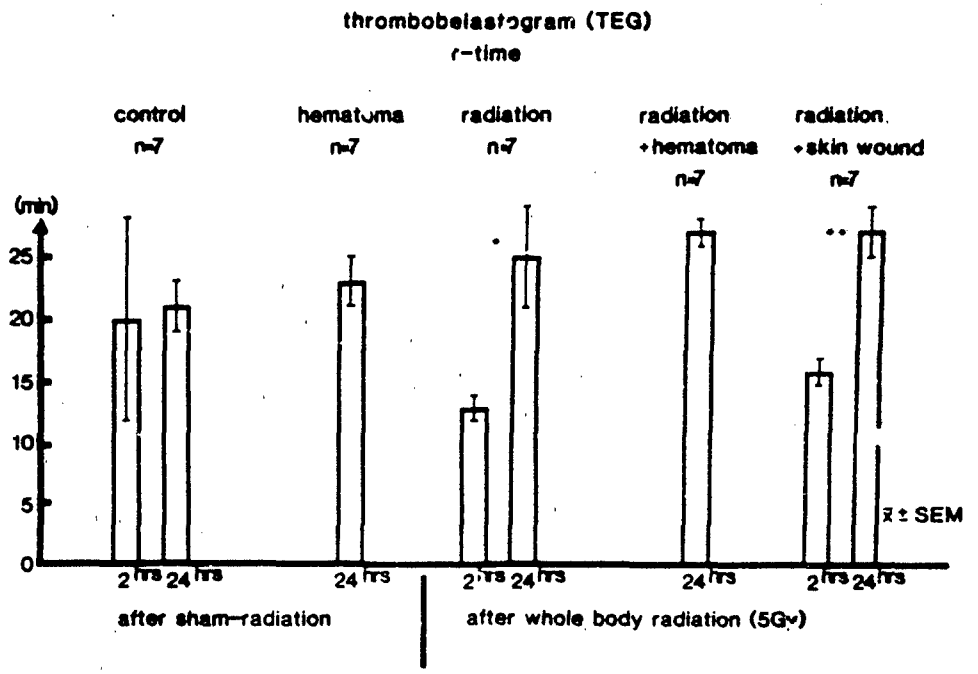


Figure 6. The r-time of the thrombelastogram after sham or whole-body irradiation with and without additional trauma. *: $\alpha < 0.05$; **: $\alpha < 0.01$.

Observations concerning the thrombocyte adhesion measured by the retention of glass-perls with the Morris test did not show any significant changes comparing the 2-hour or 24-hour values with the control group (Fig. 7). By contrast, the platelet aggregation analyzed with the method of Born et al.[13] increased significantly. At 24 hours, the irradiated groups (R) and particularly both combined-injury groups (R + H and R + W) had a significantly shorter lag time until aggregation occurred (Fig. 8). Furthermore, the aggregation time representing the reactivity of the thrombocytes decreased significantly in the irradiated group (R) and most dramatically in the combined-injury groups (R + H and R + W) (Fig. 9). At 2 hours, however, the aggregation time in the Born test was slightly increased in the radiated group (R) and most prominently in the combined injury group (R + W).

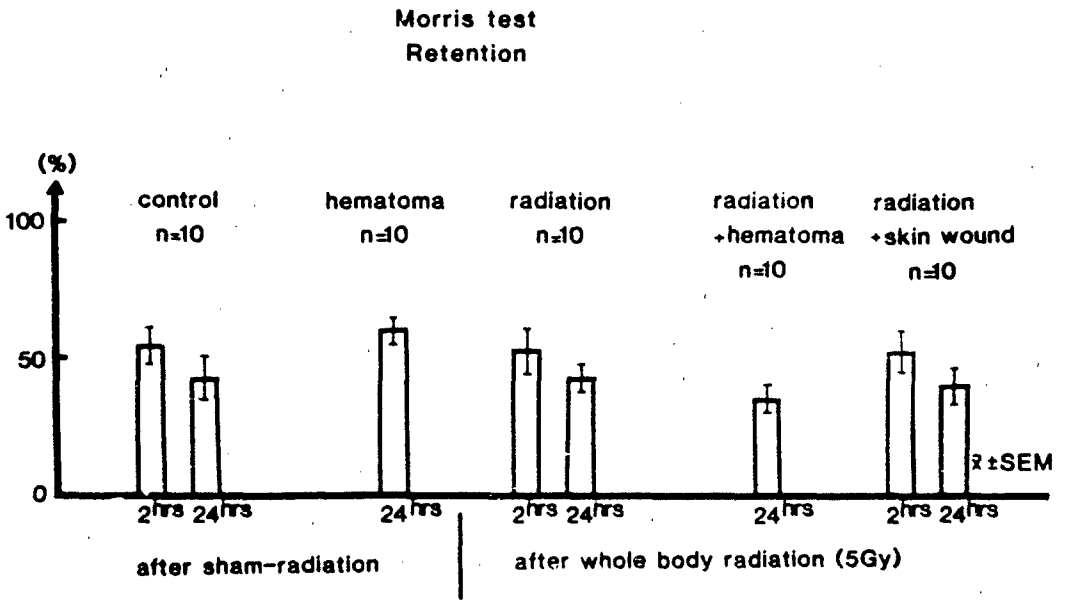


Figure 7. Platelet retention in the Morris test after sham or whole-body irradiation with and without additional trauma.

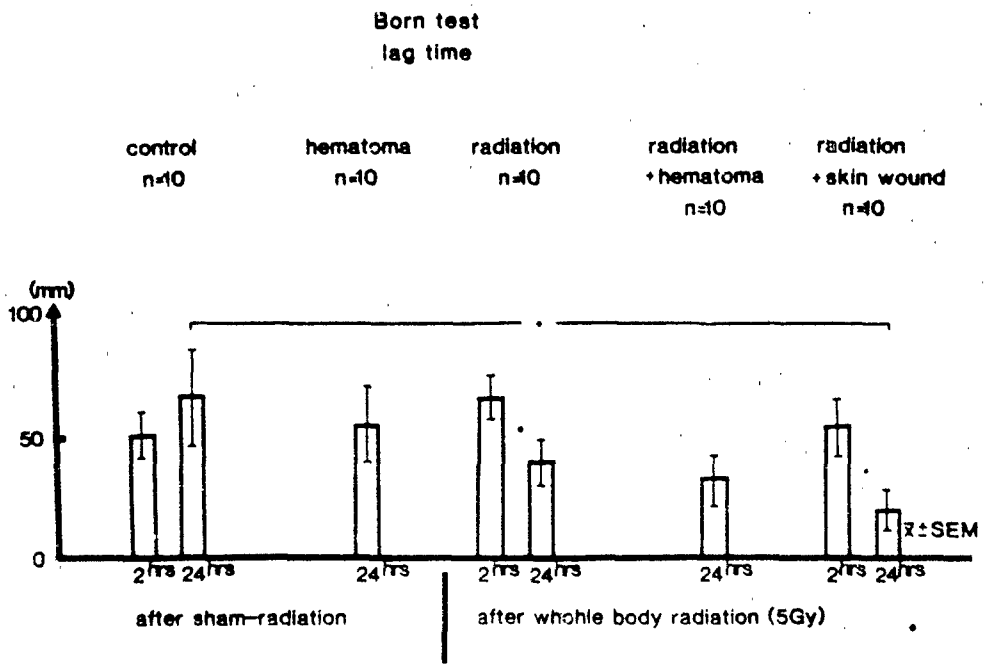


Figure 8. Lag time measured in the Born test after sham or whole-body irradiation with and without additional trauma. *: $\alpha < 0.05$.

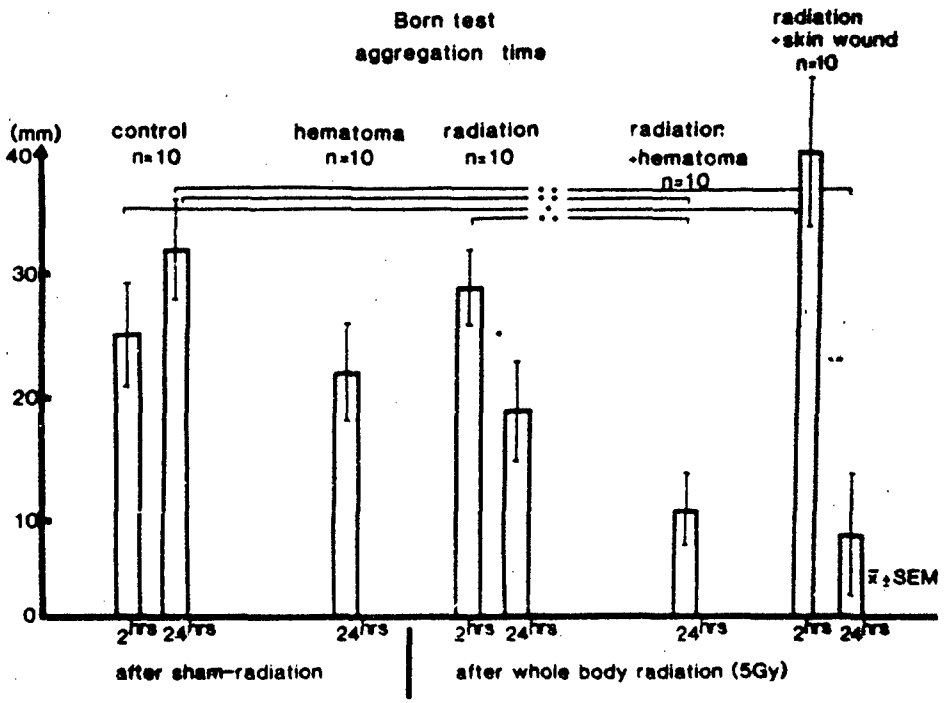


Figure 9. Thrombocyte aggregation time after sham or whole-body irradiation with and without additional trauma. *: $\alpha < 0.05$; **: $\alpha < 0.01$.

A similar pattern could be demonstrated from the total aggregation time (Fig. 10). There was a significant decrease in all irradiated groups (R, R + H, R + W) at 24 hours after irradiation. At 2 hours after radiation, however, a slight increase in the radiated group (R and R + W) was measured. The maximal amplitude after addition of collagen in the Born test gave corresponding results (Fig. 11): A significant decrease after 24 hours of radiation in all irradiated groups (R, R + H, R + W) and most dramatically in the (R + W) group. A minimal decrease could be shown, however, in the animals challenged with only a hematome at 24 hours. Similar to the aggregation time at 2 hours after irradiation, a slight increase was observed only in the (R + W) group.

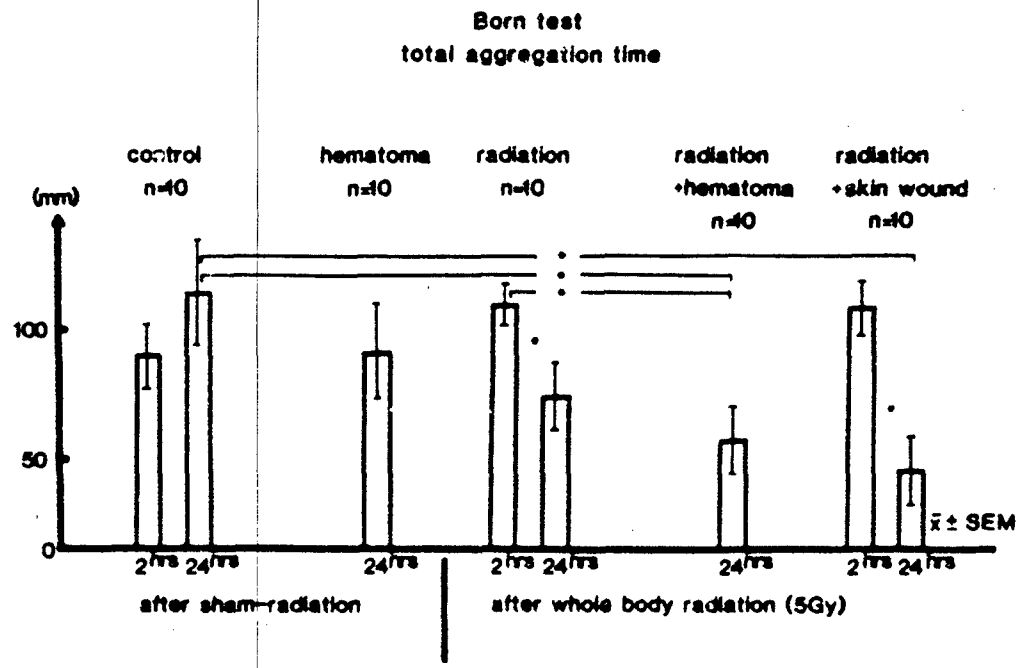


Figure 10. Total aggregation time of platelets after sham or whole-body irradiation with and without additional trauma. *: $\alpha < 0.05$.

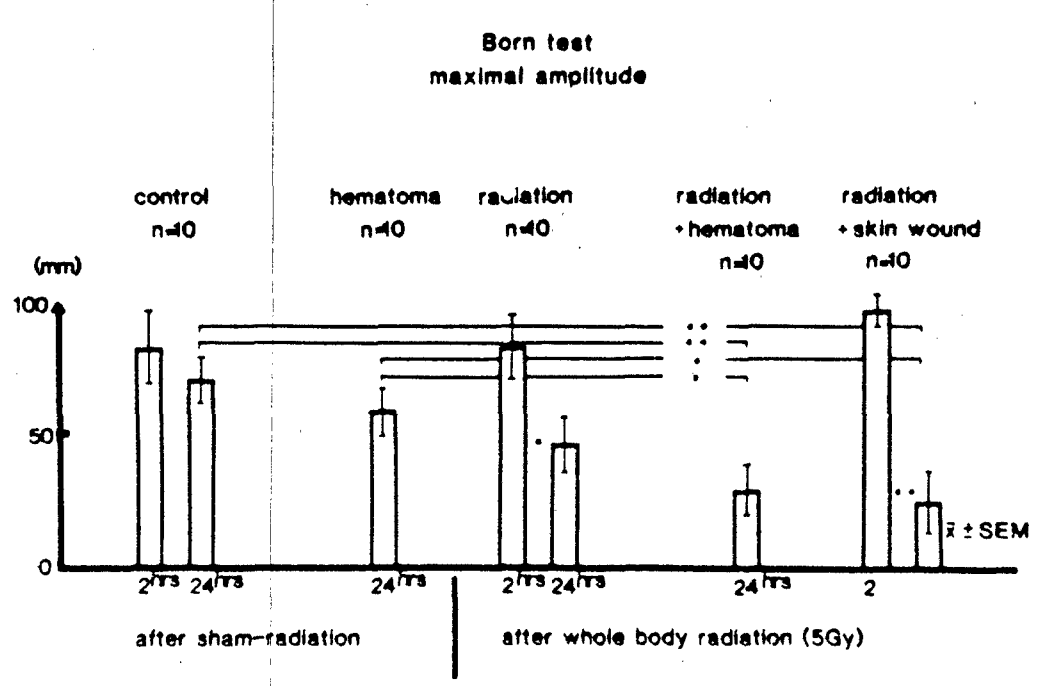


Figure 11. Maximal amplitude measured in the Born test after sham or whole-body irradiation with and without additional trauma. *: $\alpha < 0.05$; **: $\alpha < 0.01$.

DISCUSSION

A prolonged bleeding and clotting time is characteristic after whole-body irradiation.[15-18] In addition, dose-dependent thrombocytopenia damage to the whole vessel wall or the vascular endothelium[18-28] is anticipated, as well as disturbances of the plasma coagulation factors and the thrombocyte functions.

Intraoperative examinations of the plasma coagulation cascade after surgical traumas showed an increased fibrinolysis, although the procoagulatory activities were increased.[29-31] Postoperatively, both the fibrinolytic and the procoagulant activities decreased, the latter somewhat slower according to Kusin et al.[30] The diminishing of postoperative fibrinolysis is caused by a decreased content of plasmincgen activators in the blood vessels,[31-33] an increased plasminogen consumption,[31] and the traumatic stress.[34] In addition, Burkhardt et al.[35] noted an increased fibrinolytic activity and a decreased fibrinogen concentration in their animal experiments after a traumatic shock. Furthermore, the formation of clots led to thrombocytopenia.[36-39] The vasoactive and thromboplastic substances released from the platelets[38,40] increase the permeability of the vessels, causing a hypercoagulation by fibrinogen consumption and increased fibrinolytic activity. This metabolic situation may lead to a disseminated intravascular coagulation syndrome (DIC-syndrome) in close correlation to the severity of the traumatic shock.

The platelet function after trauma is controversial. Abrahamsen[41] described a shorter life span and an increased consumption of thrombocytes. However, Emmons et al.[42] and Breslow et al.[43] observed an increase of platelets after surgery as a result of an increased production of megakaryocytes. After prolonged operations, an increased platelet-clotting activity has been reported,[36,37,39,44-52] with an increased aggregation and aggregability of the thrombocytes.[46,48,49,52] The adhesion of the platelets, however, has been shown to be increased [44,47] as well as unchanged.[46]

The hypoplastic thrombocytopenia after whole-body irradiation[53] and the hemorrhagic diasthesis observed in patients exposed to nuclear fallout[18] and in animal studies[54-58] are very pronounced. In vitro the platelets show an unexpected high radiation resistance.[59,60] According to Odell et al.,[23,61]

the mature megakaryocytes are very radioresistant whereas the megakaryoblasts are extremely radiosensitive. This may explain why the platelet number is increased immediately after radiation[5,17,62] because thrombocytes are generated as long as megakaryocytes are present.[23] Schick et al.[63] measured, as have others,[64,65] the platelet number in mice after irradiation with 3.0 to 5.0 gray, and showed a slight increase immediately following the exposure, a subsequent decrease from the 2nd to the 10th day, and a reaction period until the 20th day after irradiation. Klir et al.[66] reported a decrease of the platelet number and aggregation 3 days after irradiation, and Hohage et al.[9] made a similar observation 5 days after irradiation of rats with 7.0 gray. This might be explained by a disturbed release of ADP from thrombocytes and an increase of aged platelets, which have a reduced capacity to aggregate. Correspondingly, sublethal exposure to γ-irradiation decreased adhesion and aggregation of thrombocytes.[9,23,67,68] In contrast, Bicher et al.[19] demonstrated an increased adhesion and aggregation of platelets 5 weeks following fractionated γ-irradiation in dogs with 4.5 gray.

In our experiments the clot formation measured by the r-time in the thrombelastogram (TEG) was delayed, especially in animals exposed to irradiation and an additional trauma. This correlated well with the increased APTT time. The clot hardening expressed by the k-time in the TEG did not show any significant changes in all groups. The trigger mechanism of the platelet aggregation was accelerated by irradiation or trauma alone, and was significantly increased by combined injuries. The thrombocytes must have been in a state of irritation by the different injuries, leading to a latent activation with rapid release of adenosindiphosphate (ADP), but in a reduced quantity as documented by a decline of the maximal amplitude in the Born test. Similar results have been reported in patients with trauma alone.[69] The lag time until aggregation occurs, the aggregation time, and thus the total aggregation time were all increased after trauma or irradiation. Furthermore, there was a significant increase in the experimental groups with combined injuries. The maximal amplitude, however, was already significantly reduced by trauma or irradiation alone. Very striking was the additional reduction of the maximal amplitude in the Born test after combined injuries, which might be caused by a diminished available ADP release. In summary, we were able to demonstrate an accelerated but insufficient platelet aggregation which was

very distinct after a combined injury. An exact localization of the thrombocytic defect was not possible with the global platelet function tests used in our experiments. Nevertheless, we are able to assume a storage pool deficiency with an accelerated ADP release from the damaged thrombocytes.

SUMMARY

A prolongation of the activated partial thromboplastin time was found after radiation alone, although the fibrinogen concentration, the substrate of the coagulation cascade, was increased as well. This might be explained by structural alterations within the fibrinogen molecule after radiation exposure and combined injuries. By analyzing the thrombocyte aggregation in the Born test, we were able to demonstrate a significant decrease in the lag time 24 hours after irradiation, showing the activation of platelets that may lead to a DIC syndrome. In addition, the aggregation time reflecting the speed of the platelet aggregation is decreased after 2 hours, but significantly increased after 24 hours following combined injuries.

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DISCUSSION PERIOD WITH DR. WUSTROW

DR. DeSARDI: When you refer to antiplasmin, are you referring to alpha-2 PI, alpha-1 PI, or alpha-2 macroglobulin?

DR. WUSTROW: Alpha-2 macroglobulins. Actually you measure with antiplasmin all the compounds that are inactivating the plasmins.

DR. MURANO: With respect to hemostatic changes, I think it is important to reiterate the fact that we must pay attention to which animal species we are studying since most reagents were designed to work with human plasmas including new synthetic low molecular weight substrates.

As far as the changes in the activated partial thromboplastin time, I don't believe that you observed anything different than what we saw in the dog. There was a slight prolongation, but not a significant one.

Your APTT were in the 15-20 second area. In our dog system our reagents were diluted to a point where we were generating 25-30 second clotting times, analogous to values obtained with human plasma.

It is not surprising that you do not see a change in antithrombin-III. I am presently involved in some collaborative work with Dr. Muller Berghaus at Max Planck in Giessen. He has substantial experience with the Shwartzman model in the rabbit. We have observed that in a series of about 25 animals infected with endotoxin all responded classically exhibiting the Shwartzman reaction, consumption coagulopathy, massive renal necrosis and death, yet the antithrombin-III in these animals did not change. This is very unusual. It certainly doesn't correspond to the human situation and it obviously doesn't correspond to the dog.

One other observation on fibrinogen. You had mentioned earlier that perhaps we should have looked at fibrinogen levels in the dog at two hours. My zero time sample was taken immediately post-intervention, within a period of one to two hours. As far as your analysis of the alpha-2 antiplasmin, it is interesting that you did see a reduction in the alpha-2 antiplasmin. That would probably imply that the fibrinolytic system is substantially activated, probably to the extent that you are creating split products of fibrinogen and/or of fibrin, to the point that you might actually be coating the platelet surface, thereby resulting in the platelet dysfunction that you have documented.

DR. WUSTROW: So many questions at the same time. Thank you so much.

I think it is very important to compare these two studies. First of all, you pointed out the species differences. I am very well aware of that. You worked with the dog, which has a very low LD50, whereas we worked with rats, which have a very high LD50. This is very important to bear in mind. In addition to that, you had given 1.5 gray to the animal, whereas we gave a much higher dosage, about 5 gray. This is also important if one is to compare the studies.

I agree totally with you with regard to the AT-III studies. We have not analyzed the split products. We intend to look at those.

What I wanted to stress in my talk is that beside the immunological changes, we have evidence that a type of shock phenomenon is present in combined injury animals. We have started to look at the lung functions in

those animals, looking at the effect on activity and have found significant changes in those animals challenged with combined injury.

trauma. Although some of the proteins involved in the acute-phase response have specific functions that are clearly associated with control of inflammatory processes (e.g., inhibition of proteases by antitrypsin), the functions of others are obscure, and some of the proteins probably have multiple biological effects.

Changes in individual acute-phase proteins after various types of injuries have not been extensively studied in experimental animals. Haptoglobin increases in serum within 24 hrs after whole- or partial-body irradiation of rats.[3] Induction of local inflammation by turpentine injection appears to result in the greatest increase in rats of acute-phase proteins (such as haptoglobin), compared with changes occurring after whole-body irradiation, burns, intravenous endotoxin injection, and infection.[4] Very little work has been done on the effect of combined injury on acute-phase protein levels. Simultaneous irradiation and injection of turpentine resulted in a decrease in the maximum value of acute-phase proteins studied, compared with the increase observed after turpentine injection alone.[5] Investigations of mouse serum proteins by electrophoresis after whole-body irradiation combined with open skin wounds indicated much more pronounced changes after the combined injury than after skin wounding or irradiation alone.[6]

During the last decade, researchers have tried to predict the clinical course of various diseases from the initial level or fluctuation in the levels of several of the acute-phase proteins. Progress in clinical applications of the observations has been plagued by a random approach to the investigations, with (a) study of changes in protein levels in almost all common diseases, but inadequate study of each disease, and (b) an equally diffuse investigation of multiple individual proteins, with minimal study of each protein. Of perhaps more significance than the possible utility of acute-phase protein changes as biochemical markers of various types of disease and injury is the functional significance of their altered levels, with the added possibility that alterations in one type of injury may prove beneficial, but may be an inappropriate response

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ACUTE-PHASE PROTEINS AND SYSTEMIC IMMUNITY

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INTRODUCTION

The concept of acute-phase proteins began with the study of alterations in the serum concentration of certain proteins, especially C-reactive protein, that were observed during acute inflammation. Continued study of the acute-phase response revealed that (a) serum levels of certain proteins also increase during diverse acute and chronic conditions, and (b) although most proteins increase during disease or trauma, the concentrations of several proteins often decrease during the same interval, thus defining positive and negative acute-phase proteins.[1,2] Acute-phase proteins (or reactants) can be considered, in general, as liver-synthesized blood proteins that change in response to trauma, with the most typical changes being an early increase in C-reactive protein and later changes in α_1-acid glycoprotein, α_1-antitrypsin, ceruloplasmin, fibrinogen, and haptoglobin. Conditions leading to changes in acute-phase proteins, as determined in clinical and experimental studies, include local inflammation, thermal injury, mechanical injury, radiation injury, major surgery, bacterial infection, endotoxin injection, and neoplastic growth.[2] The response pattern of individual acute-phase proteins in response to various forms of disease and trauma may differ quantitatively as well as in time course of appearance and disappearance of these proteins. However, changes in many of the proteins occur simultaneously in various types of trauma, and specific changes do not appear to be associated with a particular type of

in another type of injury. Nevertheless, concepts of acute-phase protein changes derived from studies limited to one disease or injury should be applicable to other types of trauma.

ACUTE-PHASE PROTEIN CHANGES IN CANCER AND CORRELATIONS WITH PARAMETERS OF CELLULAR IMMUNITY

Earlier clinical studies suggested the potential usefulness of alterations in the levels of acute-phase proteins, but it appeared that the essential correlations with the proteins that were needed to explore clinical usefulness were host immune status, disease extent, and clinical course. Of the diseases evaluated, malignancy presented the most optimal setting for such correlations because of (a) a relatively protracted course of many patients, allowing the study of each subject at multiple intervals, and (b) the absence of clinical inflammation in many patients, minimizing the contribution of this variable to the serum protein levels observed in the studies. These considerations defined the populations that we selected for the first study:[7] patients with solid malignancies classified as local, regional, or systemic in clinical extent, and patients clinically cured after surgical treatment of the same types of malignancies. Control populations were grouped by age and smoking habits, because of the possible influence of these variables on protein levels.[8] None of the patients had inflammatory processes or other active noncancerous diseases. The serum levels of 18 proteins were determined by radial immunodiffusion, and peripheral blood lymphocyte *in vitro* reactivity (LR) to phytohemagglutinin (PHA) was determined by quantifying tritiated thymidine incorporation. The results of this study[7] confirmed previous observations[9] of increased levels of the acute-phase proteins, α_1-acid glycoprotein, α_1-antitrypsin, and haptoglobin (and to a lesser extent, other acute-phase proteins) in tumor-bearing patients, compared with normal controls and patients clinically cured of cancer. The levels of these three acute-phase proteins appear to be quantitatively the most important changes in serum glycoproteins in cancer. Their levels increased progressively with extent of tumor, and correlated inversely with LR to PHA. Also confirmed was the previous observation of an inverse

relationship between α_2HS-glycoprotein and presence of tumor.[9] Serum levels of α_2HS-glycoprotein correlated with LR to PHA in the patient groups.

The unique relationship demonstrated between disease extent, cellular immunity, and levels of the proteins was the rationale for the subsequent studies. For these, we selected the six acute-phase proteins that showed the highest positive or negative correlations with tumor extent and immune reactivity (positive: α_1-acid glycoprotein, α_1-antitrypsin, and haptoglobin; negative: α_2HS-glycoprotein, prealbumin, and albumin).

To confirm the relation between cellular immunity and serum levels of acute-phase proteins demonstrated in the first study, the serum levels of the six proteins were measured in patients with solid malignancies who had determination of LR to PHA as well as <u>in vivo</u> quantitative delayed hypersensitivity (DH) to dinitrochlorobenzene (DNCB).[10] The patients had local or regional tumors only; they were not evaluated if they had signs of systemic illness such as fever or elevated white blood cell count. A control population consisted of healthy volunteers. Among 147 patients studied, the mean levels of the positive acute-phase proteins were significantly higher than in controls, whereas the negative acute-phase proteins were significantly lower except for albumin, which did not differ in the patient and control populations. The serum levels of haptoglobin and α_1-acid glycoprotein correlated inversely, and α_2HS-glycoprotein correlated directly with LR to PHA (Figure 1).

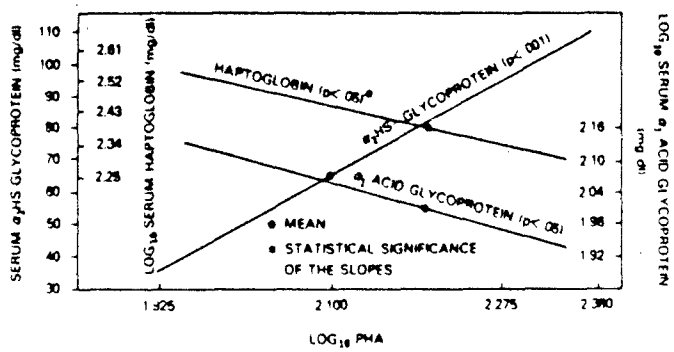


Figure 1. Regression of serum levels of haptoglobin, α_1-acid glycoprotein, and α_2HS-glycoprotein on <u>in vitro</u> lymphocyte reactivity to PHA in 147 patients with solid malignancies

To determine the relation between levels of the serum proteins and DH to DNCB, we used the method of quantitating skin test reactivity to DNCB previously described.[11] The primary immune response was scored as 4+, 3+, 2+, or 0. A 4+ and 3+ were for normal reaction, and 2+ or lesser reactions were for subnormal responses. Among the cancer patient population, the serum levels of haptoglobin and α_1-acid glycoprotein were significantly higher in patients with abnormal (2+, 0) skin test responses to DNCB than in patients with 3+ and 4+ responses (Figure 2); the levels of α_2-HS-glycoprotein and prealbumin were lower in patients with subnormal responses than in patients with normal responses to DNCB. Uniquely, the serum levels of α_2HS-glycoprotein, when grouped by DH to DNCB, showed progressive increases for each increase in skin test reactivity. Comparison of the levels of α_2HS-glycoprotein and LR to PHA grouped by DH to DNCB showed similar incremental increases in the mean levels of α_2HS-glycoprotein and LR to PHA.

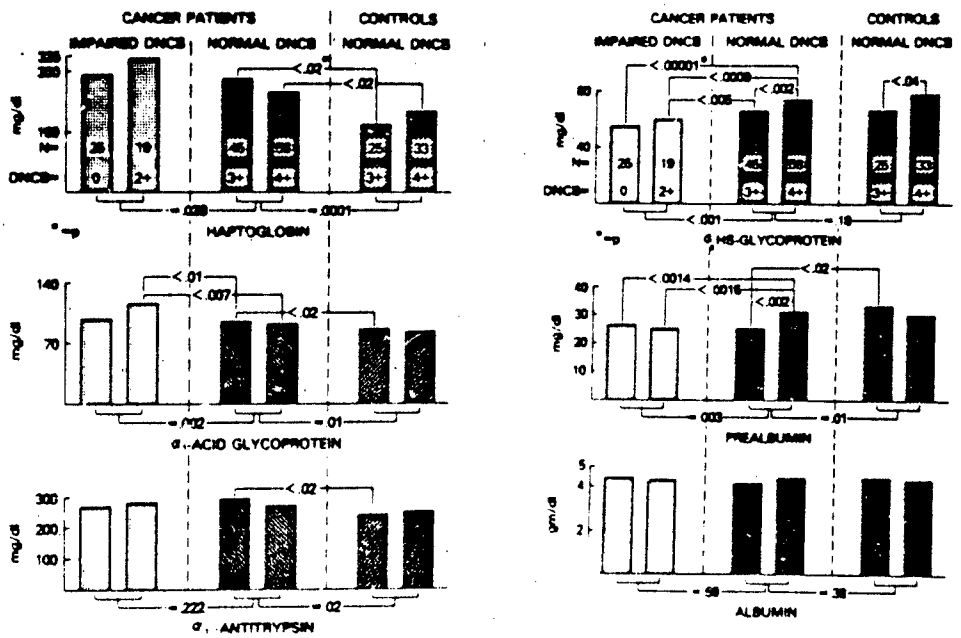


Figure 2. Median serum levels of acute-phase proteins in 147 patients with solid malignancies and in normal controls, with data grouped by delayed hypersensitivity to DNCB

IMMUNOLOGICAL PROPERTIES OF ACUTE-PHASE PROTEINS

The correlations of serum levels of some acute-phase proteins with immune status emphasize the importance of evaluation of systemic immunity, compared to cellular immune parameters, for gaining insight into clinically relevant aspects of host-disease relationships. Systemic immunity refers to the dynamic and complex interaction between all cellular and humoral factors that summate to constitute the host-disease status at any given time. This view is supported by accumulating experimental evidence on the role of acute-phase proteins in immunomodulation. Lymphocyte reactivity to PHA[12] and the mixed-lymphocyte response[13] are depressed by pure preparations of the major positive acute-phase proteins. α_1-Acid glycoprotein suppresses E-rosette formation[14] and bacterial phagocytosis by neutrophils,[15] and has a chemotactic effect on human monocytes (unpublished observations). Both *in vivo* and *in vitro* immune responses of mouse spleen cells to sheep RBC were found to be suppressed by α_1-antitrypsin,[16] and considerable evidence exists that, at the cellular level, antitrypsin is involved in lymphocyte function. Other immunosuppressive factors apparently exist in serum; these are often associated with α-globulin fractions,[17] but it is unclear how they relate to the acute-phase response. Apffel and Peters[18] have postulated that the increased levels of liver-produced sialoglycoproteins are nonspecifically immunosuppressive, perhaps binding to and protecting bystander cells during inflammatory processes. Current evidence then suggests that the increase in acute-phase proteins that are quantitatively of major importance in various types of trauma might in some way lead to immunosuppression. Because of some of this evidence, plasmapheresis has been tested as a treatment modality in cancer patients to remove increased levels of circulating acute-phase proteins. However, after one plasma exchange, acute-phase protein levels can return to pretreatment levels within a short time.[19]

Some of the proteins depressed in various types of disease and injury (α_2HS-glycoprotein, prealbumin) may have predominantly immunorestorative properties. Prealbumin has thymus hormone-like properties.[20]

α_2HS-glycoprotein promotes bacterial phagocytosis by neutrophils (opsonization).[21] This property of α_2HS-glycoprotein and an opposite effect of α_1-acid glycoprotein suggested to van Oss et al.[15,21] that one of the reasons for increased infections in trauma patients was a depressed level of α_2HS-glycoprotein and increased levels of α_1-acid glycoprotein, despite normal immunoglobulin levels. α_2HS-glycoprotein was also found to enhance the ability of mouse macrophages to take up latex particles and radiolabeled DNA.[22] This ability to partake in the clearance of foreign material and debris may be similar to some properties of fibronectin, another protein important in trauma.

EFFECT OF RADIATION THERAPY AND SURGERY ON ACUTE-PHASE PROTEINS AND IMMUNE PARAMETERS

The effect of radiotherapy on serum protein levels was investigated by considering patients being treated for localized head and neck tumors. In this study, α_1-acid glycoprotein and haptoglobin levels increased in the nontreated group, whereas α_2HS-glycoprotein was depressed. The changes were not significantly different from the normal control group (Figure 3). T-cell levels and lymphocyte reactivity to PHA were

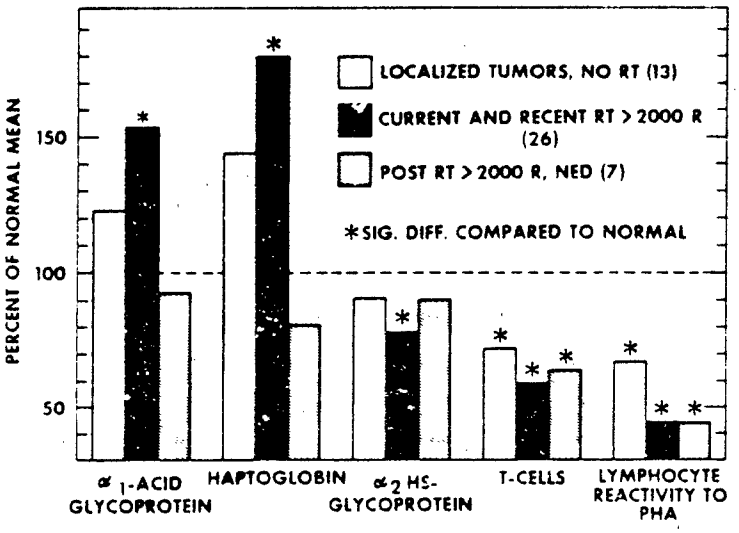


Figure 3. Effect of radiotherapy on serum glycoproteins and T-cells in patients with localized head and neck cancer

significantly depressed compared to normal. Current radiotherapy to the tumor site resulted in further increases in the positive acute-phase proteins, while α_2HS-glycoprotein, T-cell levels, and lymphocyte reactivity were further depressed. In patients with no evidence of disease after radiation therapy, the serum protein levels tended to normalize, but the T-cell levels and lymphocyte reactivity did not. Levels of α_1-acid glycoprotein were correlated on radiation dose received by the patients during radiotherapy. A significant positive correlation between protein levels and radiation dose received was observed (Figure 4). On the other hand, levels of α_2HS-glycoprotein and T-cells were depressed with increasing radiation dose (Figure 4). Correlations with radiation dose

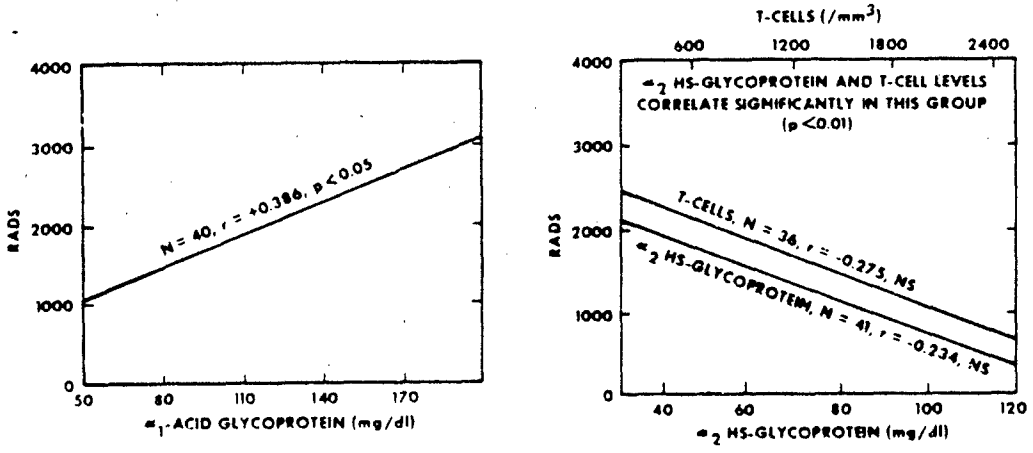


Figure 4. Correlation of levels of α_1-acid glycoprotein (left), α_2HS-glycoprotein, and T-cells (right) on radiation dose in patients during radiotherapy for localized head and neck cancer

were not significant, but levels of α_2HS-glycoprotein and T-cell levels correlated significantly in these patients. Serum protein levels eventually reflect both changes due to tumor eradication and opposite changes due to radiation injury of various tissues. Changes in immunocyte levels and function, however, may persist for weeks or months, even in cancer patients who benefit clinically from this treatment. The results may be explained by a direct effect of the irradiation on lymphocytes coursing through blood and lymphatic vessels encompassed by the radiation

portal, and by radiation destruction of the lymphocyte precursors in the marrow included in the radiation portal. Since the direct effect of radiation therapy on lymphocytes and other immunocytes may produce a long-lasting cellular immune deficiency in cancer patients cured by the therapy, cellular immunity may not correlate with the clinical course of cancer patients during successful radiation therapy. Relevant here is our finding that serum levels of acute-phase proteins may not parallel lymphocyte and T-cell levels during radiation therapy. In studies of cancer patient populations that benefit from radiation therapy, α_2HS-glycoprotein levels usually increased during tumoricidal radiation therapy[23], and levels of α_1-acid glycoprotein and haptoglobin decreased.

Surgical trauma has been shown to result in changes in many acute-phase proteins.[24] Within 6-8 hrs, C-reactive protein increased, followed by α_1-acid glycoprotein, and at 1 day, haptoglobin, antitrypsin, and other proteins increased while prealbumin and albumin were depressed. The peak period of the changes in specific proteins appeared to differ. In our study[25] of patients with brain tumors undergoing surgical treatment, significant increases were seen in α_1-acid glycoprotein and haptoglobin and a depression in α_2HS-glycoprotein before treatment, compared to normal. After surgery, levels of haptoglobin and α_1-acid glycoprotein increased to twice normal levels, and the increase persisted 2-8 weeks after surgery. Levels of α_2HS-glycoprotein and T-cells were further depressed 1 week after surgery, but tended to normalize at later times studied. These findings may have implications for the immunosuppressive effects of surgical trauma.

EVALUATION OF ACUTE-PHASE PROTEINS IN IMMUNOTHERAPY

The clinical course of cancer patients after effective treatment (e.g., interval to relapse or death) has been correlated with the results of assays of cellular immunity obtained before treatment. The correlations of α_2HS-glycoprotein with LR to PHA and DH to DNCB prompted us to investigate the relationship of serum levels of this glycoprotein with

survival after treatment for cancer. For this assessment, we used a controlled trial in small cell carcinoma of the lung. In this clinical study, patients were randomized into two groups; both received the same combination chemotherapy regimen, and one also received thymosin fraction 5. The goal of this experiment was to evaluate the effects of thymosin on patient survival, with the premise that the agent would improve survival by ameliorating the suppression of immunity due to the tumor and the chemotherapeutic drugs. In previous studies, the beneficial effects of thymosin on immunity appeared to be confined to hosts with impaired cellular immunity. Therefore, in this study, the populations were divided into groups with relatively high and relatively low cellular immunity, using T-cell level as a measure of cellular immunity and the median T-cell level as the point of division of each population into high and low groups.

Among patients with high total T-cells or α_2HS-glycoprotein levels, survival was similar among patients who received chemotherapy alone and those who also received thymosin. However, among patients with low total T-cells or α_2HS-glycoprotein levels, the survival of patients who received thymosin was significantly longer than those who received chemotherapy alone (Figure 5). Analysis by tests of interaction shows that survival correlated with low serum α_2HS-glycoprotein levels ($p = .046$) but showed only a trend for a similar association with T-cell levels ($p = .17$). The results of this study[26] provided the first correlation of serum levels of an acute-phase protein and prognosis after treatment of clinical disease. It also gave important comparisons between the results of an assay of cellular immunity and serum levels of the protein, when both were used as an indicator of survival. In view of the correlation of serum α_2HS-glycoprotein levels and assays of cellular immunity, it was plausible that the pretreatment levels of α_2HS-glycoprotein correlated with prognosis after immunotherapy. The greater association of serum levels of α_2HS-glycoprotein with prognosis compared with total T-cell levels raises the speculation that, at least under certain conditions, serum acute-phase proteins may have or reflect biological functions that are as critical in determining clinical course as cellular immune functions.

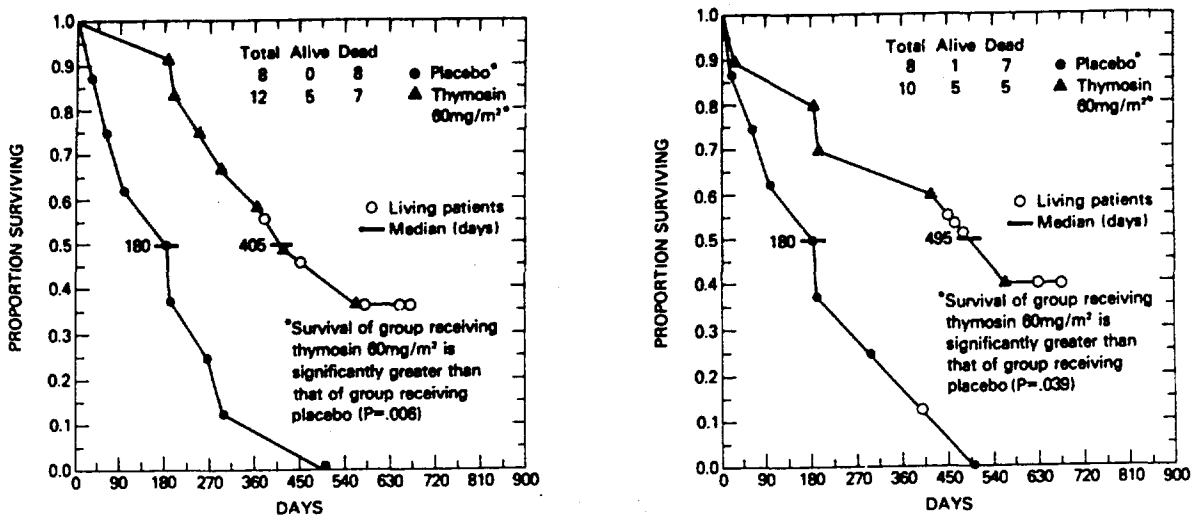


Figure 5. Life table analysis of survival of patients with low pretreatment levels of total T-cell (left) or α_2HS-glycoprotein (right), who received chemotherapy alone or chemotherapy and thymosin fraction 5

The clinical evaluation of agents that alter immune reactivity would be facilitated by assays of systemic immunity that indicate a favorable effect before the manifestation of clinical efficacy by the agents. During the evaluation of the effects of the thymic peptide α_1, patients with incurable malignant gliomas were chosen for the first clinical assessment of the agent. Preliminary assessment of a group of patients with gliomas showed a correlation of α_2HS-glycoprotein with peripheral blood T-cell levels.[25] The peptide was administered in three dose levels (300, 600, and 900 micrograms/m^2) to two, five, and three patients, respectively, who had incurable gliomas treated with surgery and palliative radiation therapy. Four additional patients received thymosin fraction 5 at 60 mg/m^2. The agents were administered twice weekly for 4 weeks. Assays of cellular immunity and quantitation of serum acute-phase protein levels were performed twice weekly for 2 weeks before administration of the agents, during the 4-week interval of administration of the agents, and for 4 weeks after. T-cell levels increased during the 2nd to 4th week of agent administration, and then either remained constant or declined toward baseline levels (Figure 6). By contrast, the levels of α_2HS-glycoprotein (Figure 6) and prealbumin increased during administration of the agents and continued to rise during the subsequent 4-week

interval. These results demonstrate the usefulness of serum factors in assessing experimental agents whose clinical effects may be associated with changes in immune reactivity.

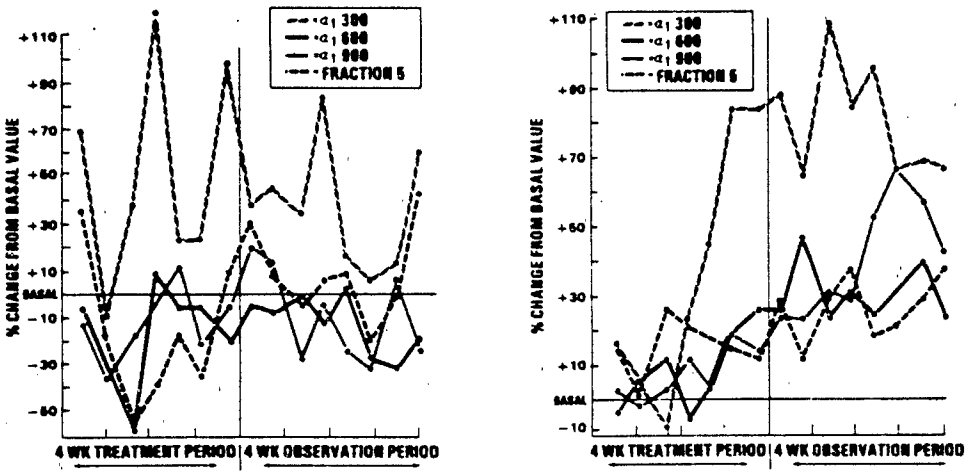


Figure 6. Median total T-cell (left) or α_2HS-glycoprotein (right) levels in patients with malignant gliomas who received thymosin fraction 5 or α_1 for 4 weeks

The effect of various immunotherapeutic agents, per se, on serum acute-phase proteins needs to be studied in detail since available studies indicate that this class of drugs may affect serum protein levels. For example, lentinan, a glucan derivative, results in increased positive acute-phase proteins and increased α_2HS-glycoprotein.[27]

SUMMARY AND CONCLUSIONS

Studies on acute-phase protein changes in various types of trauma have been reviewed here and the possible immunological implications of these changes have been discussed. The studies from our laboratories described correlations of the serum levels of certain acute-phase proteins with

several clinically relevant aspects of the host-disease relationship in cancer. Specifically, the proteins correlated with (a) primary delayed cutaneous hypersensitivity to DNCB, (b) in vitro lymphocyte reactivity to PHA and total T-cell levels, (c) tumor clinical stage and patient course after treatment, and (d) efficacy of experimental therapy regimens, in comparison with other indices of efficacy such as T-cell levels or patient survival. Since the correlations made with the proteins selected for the studies may not be obtained with other acute-phase proteins, it appeared appropriate and helpful to select a term (Immune-Reactive Proteins) to denote these and other proteins found to show similar properties in future investigations. It appears that the correlations emphasize the importance of evaluations of systemic immunity, compared to cellular immune parameters, for gaining insight into clinically relevant aspects of host-disease relationships. This view is supported by experimental evidence. Future studies should determine the most appropriate cellular and circulating factors that will define the extent of disease or injury, immunological status, and effects of treatment for various types of trauma, including combined injury.

The results of these studies appear to have broader implications for investigations into the interactions between host and disease than attempted thus far. The exploration of mechanisms that give rise to the correlations are essential for a comprehension of the underlying biologic processes. For example, the recently described evidence for tumor and white blood cell production of immunosuppressive glycoproteins[28,29] may require modification of the existing concepts concerning the function of these proteins, which are based on exclusive hepatic production of the proteins. The direct alteration of immunocyte-antigen interactions by the proteins has obvious potential for expanding the comprehension of the methods by which the proteins modify the course of disease. Another property of the proteins, the binding and transport of a variety of drugs, has been described.[30] Investigation of this property may have direct clinical applications in various forms of trauma, and may partially explain the

observed effects of chemotherapeutic agents. The direct effects of drugs, especially immunomodulators, on the synthesis of acute-phase proteins need to be studied in relation to the activity of the drugs.

The relation between the extent of tumor or clinical stage and immune reactivity as quantitated by acute-phase protein levels suggests that immune staging of diseases may provide more precise correlations with end results than is obtained with current staging schema. Thus, although few in number and preliminary in nature, the observations give certain acute-phase proteins a central role in determining the aggressiveness of disease, the integrity of host defense capabilities, and the impact of treatment on both disease and host.

Studies involving human patients at the University of Maryland and the National Institutes of Health were performed in conformity with the "recommendations guiding doctors in clinical research" as stated in the Declaration of Helsinki of the World Health Medical Association (1964).

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USE OF BLOOD COMPONENTS IN THE MANAGEMENT OF TRAUMA

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INTRODUCTION

Approximately 11 million units of blood and 2 million liters of plasma are collected each year in this country. Of 100 million potential donors, only about 3 million actually donate on a regular basis, and despite attempts to procure more donors, periodic serious blood shortages have persisted. Now, after more than 20 years of extensive evaluation of cryopreservation methods, blood banks are making use of frozen blood components to provide quality transfusion therapy when liquid blood is not available and when rare blood types are required.

When whole blood is stored at 4°C in the acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose (CPD) anticoagulant for 21 days, or in a CPD anticoagulant supplemented with adenine and additional glucose (CPDA-1) for 35 days (Figure 1), the red blood cell component has satisfactory posttransfusion survival but not satisfactory function.[1] The red cell 2,3 DPG level increases during the first 48 hours of storage, but after this time decreases at an accelerated rate (Figure 2).

Blood transfusions are given to (a) restore blood volume, (b) improve oxygen transport to tissue, (c) correct bleeding disorders related to platelet deficiency or platelet abnormalities, (d) correct bleeding disorders related to deficiency of protein clotting factors, and (e) treat specific clinical problems with gamma globulin and hyperimmune serum. It makes sense then to isolate and preserve the red cells, platelet concentrates, cryoprecipitate, fresh frozen plasma, and plasma for fractionation into albumin, plasma protein fraction, gamma globulin, and coagulation protein concentrates, and to subsequently transfuse only the specific components that are needed. A

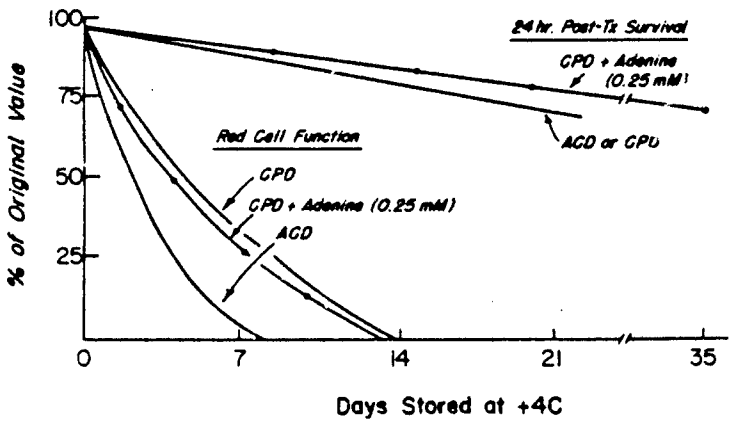


Figure 1. Twenty-four-hour posttransfusion survival and function values of red cells after storage in acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose (CPD) anticoagulant for as long as 21 days and in CPD plus 0.25 mM adenine for as long as 35 days. Red cells were stored at 4°C in ACD, CPD, and CPD-adenine anticoagulants as whole blood or as red cell concentrates, with hematocrit values of 70 V%. (From Valeri et al., Crit. Care Med., 7:440, 1979, The Williams & Wilkins Co., Baltimore, MD, with permission.

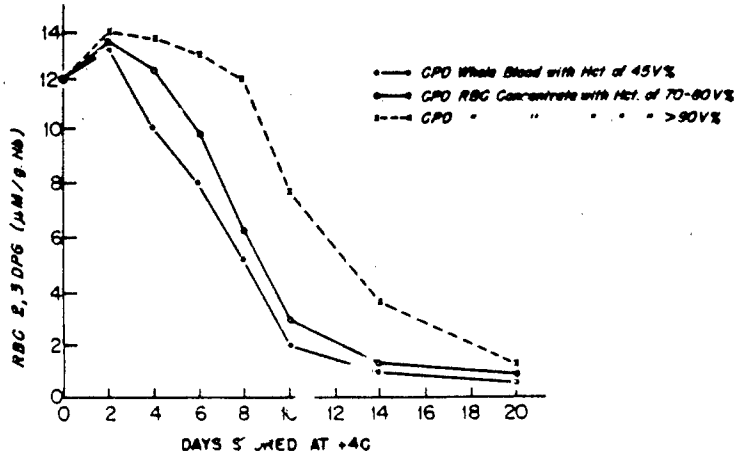


Figure 2. The 2,3 DPG levels in red blood cells stored in CPD as whole blood with a hematocrit value of 45 V% or as a red cell concentrate with a hematocrit value of 70 to 80 V% or of greater than 90 V%. Neither the whole blood nor the red cell concentrate was mixed during liquid storage at 4°C. (From Valeri, C.R., Surgical Pounds 4:41, 1981, with permission.)

plastic multiple-bag collection system allows the preparation of the various blood components--red cells, platelets, and plasma--in a closed environment without risk of contamination (Figure 3).

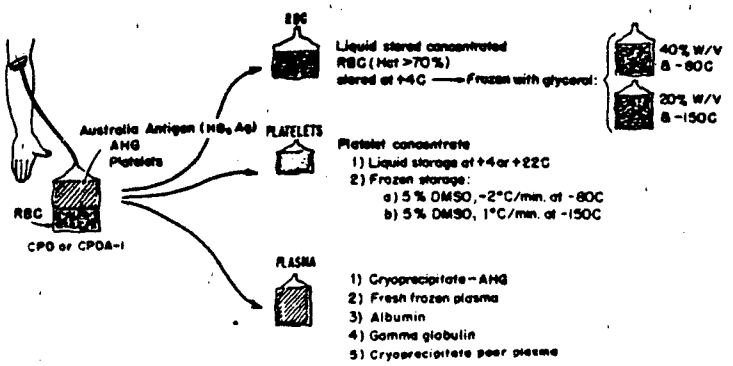


Figure 3. An approach to collecting 450 ml of blood, separating it into cellular components and plasma protein derivatives. The red cell and platelet concentrates and plasma protein derivatives are isolated from blood within 4 hours of collection and storage at room temperature (22°C ± 2°C) and are preserved by the most appropriate method. (From Valeri, C.R., Blood Banking and the Use of Frozen Blood Products, CRC Press, Boca Raton, FL, 1976, 3, with permission.)

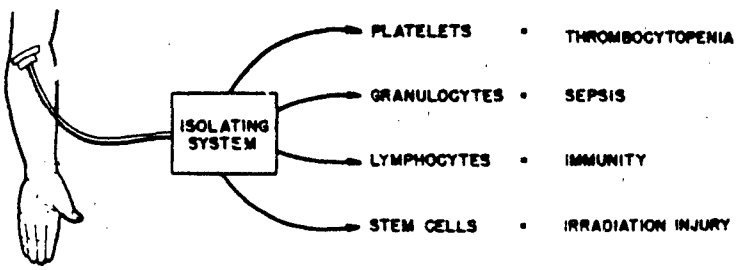


Figure 4. Scheme of how cells can be isolated from peripheral blood using the mechanical cell-separating systems to obtain platelets, phagocytic cells (granulocytes-monocytes), lymphocytes, and stem cells. (From Valeri, C.R., Crit. Rev. Clin. Lab. Sci. 14:23, 1981, with permission.)

The number of white blood cells and platelets isolated from one unit of blood is too small to be therapeutically effective, so it has been necessary to pool platelets and white blood cells from several units of blood, and this introduces the potential for infection and sensitization. However, with the newly improved methods of plasmapheresis, plateletpheresis, and leukapheresis, which are well tolerated by healthy donors, it is now possible to harvest only the specific component or components needed and return the residue to the donor (Figure 4). It is also possible now to isolate totipotential hematopoietic mononuclear stem cells from human peripheral blood.[2-10]

INDICATIONS FOR BLOOD TRANSFUSION

Blood transfusions are given primarily to restore blood volume and to improve oxygen transport to tissues, and the donor red cells must have adequate circulation property in order to increase the oxygen-carrying capacity.[1]

Stored whole blood has been used extensively to treat hypovolemia associated with hemorrhagic shock and to prevent this condition from occurring during surgery associated with blood loss. As blood is stored at 4°C, the viability and function of platelets and granulocytes and of the labile plasma protein clotting factors deteriorate, and microaggregates form. The albumin, gamma globulin, and fibrinogen remain stable at 4°C for up to 21 days.[1] Red cell concentrates are now being used in place of whole blood to treat hemorrhagic shock because they have comparable posttransfusion survival values and better oxygen transport function (Figure 2).[1] Liquid-stored red cell concentrates with hematocrit values of about 75 V% contain only one third as much fibrinogen, gamma globulin, and albumin, and less citrate and extracellular potassium ion than liquid-stored whole blood. Washed previously, frozen red cell concentrates have been used in combination with crystalloid and colloid plasma volume expanders in the treatment of hemorrhagic shock, with satisfactory results.[11]

Moderate to severe hemorrhagic shock produces a decrease in perfusion of organs which, in turn, leads to acidosis and tissue hypoxia. Usually, treatment consists of infusions of (a) crystalloid and colloid to restore the plasma volume and the interstitial volume and (b) red cells to restore the red cell volume and the oxygen delivery capacity of the blood.[12-26] Platelets and coagulation factors also may be required later.

The physical condition of the recipient will largely determine which blood products will be administered and in what quantity. The nature and extent of the primary illness or injury, the amount of blood and fluid losses, the patient's age and previous state of health, the number and extent of associated medical conditions, the time delay in instituting therapy, and the course of immediate therapy must all be considered.

Whole blood, either fresh or liquid-preserved, often is used to resuscitate moderate-to-severe hemorrhagic shock.[27,28] Actually, it is the red cell component of the blood and not the plasma that is needed during moderate-to-severe hemorrhagic shock, and red cells used in combination with crystalloid solutions have been shown to be as effective as whole blood.[29-31] The red cells do not have an oncotic effect in vitro; however, they do produce an in vivo increase in plasma volume (apparently by the mobilization of interstitial albumin). They produce an immediate increase in red blood cell volume, followed by a prompt and satisfactory increase in plasma volume.[31] It is important that the transfused red cells have satisfactory oxygen transport function, i.e., normal or increased 2,3 DPG levels at the time of transfusion, so that an ample supply of oxygen will be delivered to tissues.

PHYSIOLOGIC IMPORTANCE OF OXYGEN TRANSPORT FUNCTION OF PRESERVED RED CELLS

It has been more than 25 years since the impairment of oxygen transport function in liquid-stored red cells was first described,[32] and almost 15 years since this respiratory defect was linked to the deterioration of red cell 2,3 DPG during storage.[33,34] Because this impairment usually is corrected in vivo

within 24 hours after transfusion, and because it does not critically affect many patients, it has gone unnoticed by many physicians.[1,35] However, this phenomenon is an important one because the rate of 2,3 DPG restoration is affected by the acid-base status, degree of anemia, cardiopulmonary function, plasma inorganic phosphorus level, and other factors.[35] Studies have shown that oxygen transport function is especially critical in patients with fixed cerebral and coronary blood flow, hypothermic patients in hemorrhagic shock, and hypothermic patients undergoing cardiopulmonary bypass surgery, and such patients should be given red cells with increased 2,3 DPG levels and improved oxygen transport function.[35]

From 1968 to 1972, the Naval Blood Research Laboratory was involved in studies of more than 300 patients who had sustained war injuries in South Vietnam and were being treated at the Chelsea Naval Hospital (later named Boston Naval Hospital, Chelsea, MA). The use of general anesthesia for routine debridement of wounds precipitated a life-threatening state of hypotension in these patients. The body's compensatory mechanism for the reduction in red cell volume was to increase the red cell 2,3 DPG levels to ensure optimum oxygen transport function without an increase in cardiac output. A 40% reduction in the red cell volume usually was compensated for by an increase in the level of 2,3 DPG from 0.8 moles DPG/mole Hb or 13 uM/g Hb to 1.6 mole DPG/mole Hb or 25 uM/g Hb, which is two times the normal level.[31]

Our studies in these patients led to the development of a rejuvenation solution, now called PIPA, containing pyruvate, inosine, phosphate, and adenine, which produces these types of elevations in red cell 2,3 DPG levels in vitro. Red cells that are biochemically treated with PIPA solution after 6 to 8 days of storage at 4°C have 2 to 3 times normal 2,3 DPG levels and improved oxygen transport function upon transfusion. Rejuvenated red cells have been used with considerable success in treating anemic patients with coronary and cerebral insufficiency and hypothermic patients undergoing cardiopulmonary bypass surgery.[35]

PLATELET TRANSFUSIONS

Patients with dilutional thrombocytopenia associated with a bleeding diathesis may require platelet as well as red cell transusions, and the platelets must have satisfactory viability and function.[1,36,37] The shelf life of platelets is considerably shorter than that of red cells (5 days at 22°C and 2 days at 4°C), but cryopreservation with DMSO permits storage at -80°C for 2 years and at -150°C for at least 3 years.[1,36,38].

When platelets are stored at 4°C for 24 hours, they have excellent hemostatic effectiveness during the 4- to 8-hour posttransfusion period. The effectiveness of platelets that have been stored at 22°C for 5 days is impaired during the first 3 hours after transfusion; however, these platelets do have excellent posttransfusion survival.[1,36,38] When platelets are frozen within 6 hours of collection, either with 5% DMSO and storage at -150°C or with 6% DMSO and storage at -80°C, they have excellent hemostatic effectiveness. But post-transfusion survivals are only about half those of fresh platelets, so two units are needed to achieve an increase in platelet counts comparable to that achieved with one unit of fresh platelets.[1,36]

PLASMA OPSONIC PROTEINS

The opsonic plasma proteins in blood (which consist of immunoglobulins, IgG, IgM, and IgA complement, and plasma fibronectin, a cold insoluble globulin[39-42]), are thought by some to be beneficial in the prevention and treatment of sepsis following hemorrhagic shock, although this has not been substantiated. Cryoprecipitate containing plasma fibronectin and fibrinogen, Factor VIII, and von Willebrand's factor has been recommended for treating patients with a deficiency of plasma fibronectin following surgical procedures, traumatic injuries, and burns.[43-46] It is believed that ten units of cryo-precipitate are sufficient to treat the deficiency of plasma fibronectin. This area of research, if successful, represents a significant advancement in the treatment of acute hemorrhagic shock and prevention of septic shock. Hyperimmune serum produced to mutant Escherichia coli has been reported to be therapeutically effective in the treatment of Gram-negative bacteremia and shock in man.[47]

Studies are in progress to isolate, purify, and cryopreserve human mononuclear cells in peripheral blood; mononuclear cells can now be salvaged from the cellular residues obtained during plateletpheresis procedures. Universal donor toti-potential hematopoietic stem cells devoid of immunocompetent cells are needed to treat irradiation injury and combined injury.[48-50] Obtaining totipotential hematopoietic stem cells from the blood by means of a blood cell separator is preferable to a bone marrow harvest because the leukapheresis procedure is well-tolerated and can be performed repeatedly on an outpatient basis without anesthesia and without the discomfort associated with multiple bone-marrow punctures. The mononuclear cells can be cryopreserved with 5% DMSO and 6% HES in Normosol-R and albumin, frozen at 2-3°C per minute by storage in a -80°C mechanical freezer, and stored at -80°C or -150°C.[51,52] Cell washing is necessary to remove the DMSO and HES.

CFU-GEMM (granulocyte-erythrocyte-macrophage-megakaryocyte) and CFU-GM (granulocyte-macrophage) colonies can be grown in tissue culture, and monoclonal antibodies can be utilized to identify helper and suppressor T cells, B cells, monocytes, and non-T and non-B null-type cells.

POTENTIAL RISKS OF BLOOD TRANSFUSION

The major risk in transfusing blood products to treat acute hemorrhagic shock is the transmission of hepatitis, although testing for the hepatitis B surface antigen has reduced this risk considerably.[1] Some investigators believe that the incidence of posttransfusion hepatitis can be reduced even further if the plasma is removed from the blood before transfusion. Red cell concentrates that are washed before transfusion are relatively free of plasma. Although red cell washing does reduce the dose of virus associated with the hepatitis, it does not eliminate the transmission of hepatitis. Testing of donor blood for alanine aminotransferase has been recommended as a method to reduce the transmission of non-A-non-B posttransfusion hepatitis.[53]

Blood products may also transmit malaria, cytomegalovirus, acquired immune deficiency syndrome (AIDS),[54] and other infectious diseases, and the risk of red cell incompatibility must always be considered. The transfusion

of incompatible blood very likely produces a hemolytic transfusion reaction, resulting in renal insufficiency and disseminated intravascular coagulation. Red cell incompatibility rarely occurs as the result of failure to detect incompatible donor blood by the crossmatching procedure, but rather occurs during transfusion when, say, Group A, B, or AB blood is given erroneously to a Group O recipient. On the other hand, non-hemolytic transfusion reaction occurs when the patient has a reaction to white blood cells, platelets, plasma proteins, and non-protein substances in the blood as a result of previous allo-immunization to these substances. Other potential risks from transfusions of blood products include citrate intoxication, ammonia intoxication, amorphous debris (microaggregates in blood), and the toxic effects of the anticoagulant used for blood collection and preservation.[1]

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DISCUSSION WITH DR. VALERI

DR. KAPLAN: From a practical standpoint, or from a financial standpoint, it costs two to four times as much to use blood components. If I have a patient that I know needs red cells because of loss, or who may also need albumin or other factors, it will cost the same for a unit of plasma or whole blood. A unit of plasma and a unit of packed cells costs twice as much as a single unit of whole blood. Could you comment?

DR. VALERI: Cost-effectively speaking, you are absolutely right. If you break blood into its components, the blood banking community then is able to charge for the individual components, the cost of which is greater than a unit of whole blood. If you are looking for maximum benefit for lots of patients other than your specific burn patient, I think that you would agree the components are what we should strive for.

DR. CATRAVAS: There have been some attempts made to encapsulate hemoglobin into special liposomes as artificial oxygen carriers. Would you care to comment?

DR. VALERI: We have looked at stroma-free hemoglobin. We are also looking at fluorocarbons. Fluorosol, now being used clinically, has been beset with a number of problems. I believe the major problem is complement activation. To receive fluorosol you are required to receive 60-100 percent inspired oxygen, but before they give it to you there are some people who also suggest that you be given 2 grams of corticosteroids to avoid the activation of the complement that occurs from detergent pluronics.

If you could have a resuscitative fluid that carries oxygen, I think it would be a tremendous benefit to everybody. The reality is that you have to look at the data right now. They arn't very optimistic for either the fluorosols or the stroma-free hemoglobin.

DR. MacVITTIE: We have been working for a number of years in trying to separate the pluripotent stem cell from both the canine and the primate models in peripheral blood. We have heard rumors that your group has isolated the pluripotent stem cell from the periperal blood. Would you care to comment on your progress?

DR. VALERI: Our enthusiasm is predicated on the fact that in our fantasy, we can grow stem cells in the form of GEM, but that may not be true.

Our purification procedures, using Congo 4 elutriation, have resulted in the identification of a cell that has a volume of about 200 cubic microns. In tissue culture, these cells grow very actively into GEM. Along with the GEM, unfortunately, are a lot of other cells. Therefore, we are very interested in trying to further purify them without getting involved in sheep red cells, lectins, etc.

I think the assignment is very fundamental, and use of tissue culture to identify potential stem cells is the key.

What we would like to be able to tell you a year from today is that reconstitution after total-body irradiation is related to an in vitro system. At the present time I don't think either the GEM or Dexter culture would allow us to make that prediction.

DR. MacVITTIE: What species are you working with?

DR. VALERI: We are presently working with baboons and humans. All of the data we are now working on with regard to isolation is from peripheral blood and bone marrow of humans or baboons.

DR. MacVITTIE: Have you established the Dexter long-term cultures in the baboon or the primate?

DR. VALERI: Yes. Our cultures are primarily from baboons and humans.

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PLASMA EXCHANGE THERAPY IN PATIENTS FAILING TO RESUSCITATE FROM BURN SHOCK

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INTRODUCTION

Although burn shock almost invariably accompanies major thermal injury, it is rarely refractory to fluid resuscitation. Baxter has stated that 95% of children and 80-90% of adults will achieve resuscitation with fluid volumes predicted by the Parkland formula.[1] This formula is the most liberal of those commonly employed to guide resuscitation in the treatment of burn shock, and many patients resuscitate successfully with significantly smaller volumes of fluid.[2] However, in select patients, failures of burn shock resuscitation still occur despite administration of massive volumes of fluid. These patients include those at the extremes of age and those with exceptionally extensive tissue trauma or major electrical injuries, major inhalation injury, delay in initiating adequate fluid resuscitation, or underlying disease that limits metabolic and cardiovascular reserve.[3] In these patients, refractory burn shock and resuscitation failure remain major causes of early mortality despite advances in emergency care and transport, resuscitation regimens, and physiologic stabilization.

Numerous reports suggest that serum from thermally injured patients and animals contains circulating "factors" toxic to cellular function and presumably responsible for the phenomenon of burn shock.[4,5] Preliminary investigations utilizing plasma exchange in burn patients not responding to conventional management have demonstrated marked improvement in several clinical settings, including refractory resuscitation failure, respiratory failure in the absence of inhalation injury, myoglobinuria associated with massive electrical injuries, and the syndrome of metabolic exhaustion or "pre-sepsis."[6] In this report, the use of plasma exchange was evaluated retrospectively in a larger group of patients with major thermal injuries who failed to respond to conventional fluid volumes during resuscitation from burn shock.

MATERIALS AND METHODS

Patient Population. During a 3-year period, 22 patients underwent plasma exchange during shock resuscitation. The mean age was 22.7 years (range 1-60). The mean total-body surface area (TBSA) burn was 47.9% (range 25.5-100) with a mean full-thickness TBSA injury of 31.9% (range 3.5-88). The patient group consisted of 14 adults (all male) and 8 children (5 male, 3 female). The etiology of tissue injury was flame burns in 16 patients, electrical injuries in 3 patients, and scald burns in 3 patients. Twelve patients suffered concomitant inhalation injuries as documented by ^{133}Xenon perfusion scan.[7] Significant pre-existing medical conditions were present in 5 patients; they include substance abuse (2), hypertension and chronic alcoholism (1), congestive heart failure with chronic lung disease (1), and iatrogenic Cushing's syndrome after bilateral adrenalectomy (1).

Resuscitation Protocol. Initial management of each patient included the routine placement of a nasogastric tube, foley catheter, arterial line, and two large-bore intravenous catheters for fluid infusion. Fluid resuscitation was initiated according to Parkland formula guidelines (4 cc Ringer's lactate solution/kg body weight/% TBSA burn in the first 24 hours).[8] However, this formula was employed merely to estimate fluid deficits and requirements. Once resuscitation was instituted, standard clinical guidelines were used to assess the adequacy of fluid resuscitation, the primary criterion being maintenance of adequate urine output. Fluid infusions were adjusted hourly to maintain a minimal acceptable urine output defined as 30 cc/hr in adults and 1 cc/kg/hr in children. For patients sustaining electrical injuries, the minimal acceptable urine output was 75 cc/hr in adults and 2 cc/kg/hr in children. Patients transported to the burn center had fluid resuscitation initiated prior to transport. Patients who failed to maintain urine output were given increasing volumes of crystalloid as needed until they had received twice the volume predicted by the resuscitation formula. At this time, patients were switched to a resuscitation regimen based upon hypertonic lactated saline (180-230 meq Na/liter) in an attempt to restore urine output.[9] Hypertonic fluid was initially infused at the rate predicted by the resuscitation formula, and subsequently titrated to urine output.

Indications for Plasma Exchange. In 17 patients, the indications for plasma exchange were ongoing fluid requirements exceeding twice those predicted by Parkland formula guidelines despite conversion to hypertonic lactated saline resuscitation fluid. In an additional 5 patients, plasma exchange was performed for massive myoglobinuria in addition to resuscitation failure. Patients failing to respond to fluid resuscitation also demonstrated persistent lactic acidemia, depressed mental status, and arterial hypotension. In most patients, the diagnosis of resuscitation failure could not be made for at least 12 hours post-burn, during which time the patients often required fluid volumes in excess of those predicted by the entire 24-hour Parkland calculation. The decision to perform plasma exchange was not made until the above criteria were satisfied. The procedure was often instituted as a "last resort" in an attempt to salvage the patient from irreversible burn shock.

Plasma Exchange Protocol. The plasma exchange procedure involved performing plasmapheresis in adult patients and simultaneously replacing cc for cc 1.5 times the patient's calculated blood volume with type-specific fresh frozen plasma (FFP). The blood volume for adult males was estimated to equal approximately 7 percent of their total body weight in kilograms. For females and children, the blood volume estimate was 6 percent of their total body weight. Fluid resuscitation with Ringer's lactate solution was continued throughout the course of plasma exchange, and was modified according to urine output as outlined above.

The plasma exchange procedure was accomplished using either the IBM Model 2997 Continuous Blood Cell Separator (IBM Systems, Endicott, NY) or the Haemonetics Model 30 Discontinuous Blood Cell Processor (Haemonetics Inc., Natick, MA). The continuous cell separator has been used almost exclusively since 1981 because it is particularly well suited for patients with elevated hematocrits and hyperviscosity (i.e., hemoconcentration during burn shock). This machine separates whole blood into major components (erythrocytes, platelet-rich plasma, platelet-poor plasma, and leukocytes) with the advantage over discontinuous cell separators of requiring virtually no initial volume from the patient to "prime" the device (100 cc of normal saline is utilized). A double vascular access technique was employed. Blood was removed through a 12- or 14-gauge femoral venous catheter (Intracath^{R}, Deseret, Sandy, UT) and

directed into the centrifuge. The platelet-poor plasma fraction was removed and collected in sterile bags for research purposes. The remaining fractions were recombined with FFP and returned to the patient through a 16-gauge femoral or central venous catheter.

Exchange Transfusion Protocol. Exchange transfusions were performed in children under 5 years of age using whole blood and platelets in an isovolemic fashion. Again, the volume of exchanged fluid was calculated to be 1.5 times the circulating blood volume as defined above. Blood was removed from the patient through a femoral arterial catheter in 30-cc to 50-cc increments, with the simultaneous infusion of an equal volume of cross-matched whole blood through a femoral or central venous catheter. Six to eight pooled platelet packs were administered at the end of the procedure to replenish the anticipated platelet deficit. Throughout both plasma exchange and exchange transfusion procedures, intake and output data were carefully balanced to ensure that the procedures were isovolemic. Each procedure was performed only once in each patient during the resuscitation period.

Completion of Resuscitation. Completion of burn shock resuscitation was defined as (1) ability of the patient to maintain adequate urine output for 2consecutive hours at a rate of fluid infusion equivalent to calculated maintenance requirements; (2) resolution of lactic and metabolic acidosis; (3) stable vital signs, and (4) return of mental status of baseline, unless associated injuries, premorbid condition, or anesthesia precludes accurate evaluation. Maintenance fluid requirements are elevated in patients with major burns and were predicted by the following formula:

Evaporative water loss from the burn wound:[10]
(25 + % TBSA burn)(BSA m^2) = cc/hr*

Other maintenance fluid requirements (urine, stool, respiratory loss, etc):
(62.5 cc)(BSA m^2) - cc/hr*

Thus, total maintenance fluid requirements equal evaporative + insensible loss:
(87.5 + % TBSA burn)(BSA m^2) = cc/hr*

* BSA = body surface area calculated from the nomogram of Dubois.[11]

Monitoring. In all patients, vital signs (heart rate, blood pressure, respirations, and temperature), sensorium, urine output, and fluid intake were recorded hourly. Measurement of the following laboratory parameters were made at 4-hour intervals throughout the course of fluid resuscitation and plasma exchange therapy: complete blood count, differential, and platelet counts; serum prothrombin and partial thromboplastin time (PT, PTT); serum electrolytes, blood urea nitrogen (BUN), creatinine, lactic acid, albumin, and total protein; and arterial blood gases (ABG's). Transient decreases in systemic blood pressure were occasionally noted; however, slowing the rate of removal of blood to be exchanged resulted in a rapid normalization of blood pressure.

Statistical Analysis. Data are presented as a mean $\pm$ standard error of the mean. Student's paired t-test was used for the evaluation of paired determinations in group sampling intervals. P values less than 0.05 were considered significant.

RESULTS

The patients had a mean delay in initiating adequate fluid resuscitation of 3.0 hours (range 0.5-12 hours). Plasma exchange was performed at a mean time of 17.0 hours post-burn (range 7-48 hours). The plasma exchange procedure required a mean 4.3 hours to complete, while exchange transfusions required 1.0 hour. Sixteen patients underwent plasma exchange, and exchange transfusions were performed in 6 patients. A therapeutic response was documented in 21 of the 22 patients (95.4%). This response was characterized by a sharp decrease in fluid requirements, from a mean of 260% above the predicted hourly volume by the resuscitation formula to within calculated requirements at a mean time of 2.3 hours (range 0-8 hours) following plasma exchange. Rapid clearing of pigment was seen in the 5 patients with myoglobinuria. Only one patient, with a 100% TBSA burn (88% full-thickness injury), failed to respond to plasma exchange and expired at 18 hours post-burn. The overall in-hospital mortality for the patient group was 27.3%. Three early deaths occurred within 5 days of injury (one each due to

irreversible burn shock, cerebral hemorrhage, and respiratory failure), while 3 late deaths (at 22, 41 and 55 days post-burn) were attributed to sepsis. No major complications were associated with the plasma exchange procedure.

The patient group achieved resuscitation at a mean time of 27.3 hours post-burn (range 14-52 hours) (Table 1). The mean resuscitation volume was 6.77 cc/kg/% burn (range 1.3-15) with sodium load of 1.24 meq Na/kg/% burn (range 0.4-2.7). Plasma exchange resulted in a dramatic and statistically significant decrease in resuscitation volume and increase in urine output when comparing group means pre- and post-plasma exchange ($p < 0.001$).

TABLE 1. RESUSCITATION CHARACTERISTICS

Total resuscitation received:		
Mean duration		27.3 hours (range 14-52)
Mean fluid volume		6.77 cc/kg/% burn (range 1.3-15)
Mean sodium load		1.24 meq Na/kg/% burn (range 0.4-2.7)
Effect of plasma exchange on resuscitation volume:	Pre	0.39 ± 0.04 cc/kg/% burn/hr
	Post	0.17 ± 0.02 cc/kg/% burn/hr*
Effect of plasma exchange on urine output:	Pre	1.04 ± 0.13 cc/kg/hr
	Post	3.26 ± 0.71 cc/kg/hr*

* $p < 0.001$

Figure 1 illustrates the effect of plasma exchange on resuscitation volume. Immediately prior to plasma exchange, the mean volume of resuscitation fluid necessary to maintain a minimum acceptable urine output was 260% of the level predicted by the Parkland formula based upon burn size and body weight in kilograms. However, plasma exchange permitted a sharp decrease in fluid resuscitation volume from a mean of 0.39 cc/kg/% burn/hr (S.E. ± 0.04) to 0.17 cc/kg/% burn/hr (S.E. ± 0.02, T = 8.08, p 0.001) within 2 hours after the procedure was completed. Plasma exchange resulted in a brisk diuresis (3.26 cc/kg/hr, S.E. ± 0.71, T = 4.17, p 0.001) despite a marked decrease in fluid volume administration.

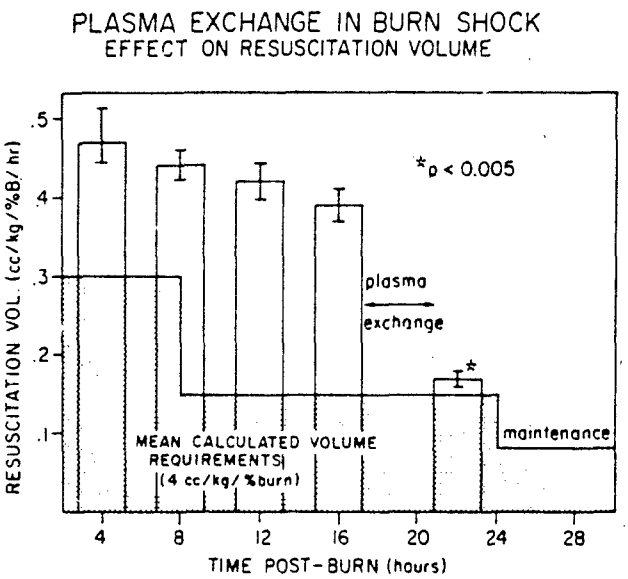


Figure 1. Effect of plasma exchange on resuscitation volume. The mean ± S.E.M. resuscitation volume for entire patient group (N = 22) is depicted on ordinate in cc/kg/% burn/hr versus time post-burn in hours on abscissa. Plasma exchange was performed at a mean time post-burn of 17 hours, required approximately 4 hours to complete, and resulted in a statistically significant decrease in fluid requirements approaching the volume predicted by the resuscitation formula.

Plasma exchange resulted in a statistically significant decrease in serum lactate levels within 2-4 hours (see Table 2). Significant increases in serum bicarbonate, pH, and base excess levels were seen immediately post-plasma exchange as metabolic acidosis resolved. No significant effect was observed on serum sodium concentration. However, a significant increase occurred in serum albumin levels.

TABLE 2. EFFECT OF PLASMA EXCHANGE ON LABORATORY PARAMETERS

	Pre-Plasma Exchange Mean	Post-Plasma Exchange Mean
	(Group mean, N = 22)	
Lactic acid (meq/l)	5.99 ± 0.728	2.76 ± 0.854*
Bicarbonate (meq/l)	20.0 ± 1.2	26.9 ± 1.0*
pH	7.40 ± 0.02	7.48 ± 0.01*
Base excess	-2.98 ± 1.3	+2.98 ± 0.8*
Sodium (meq/l)	141	144
Albumin (meq/dl)	1.69 ± 0.15	2.10 ± 0.08*

* $p < 0.001$

The colloid content of the replacement fluid (FFP) was measured and compared to the mean total protein and albumin content of burn plasma removed by plasmapheresis (Table 3). Plasma exchange resulted in a mean net gain in colloid of 131 gm of total protein and 80 gm of albumin to the patient.

TABLE 3. COLLOID EFFECT OF PLASMA EXCHANGE

7 Samples of "Normal" Fresh Frozen Plasma - Mean Colloid Levels:

Total protein - 6.29gm/dl
Albumin - 3.73gm/dl

16 Plasma Exchange Patients:

Mean Amount of Plasma Exchanged - 4858 ml
Mean Net Gain in Colloid:

Total Protein - 26.9 gm/liter exchanged
or
131 gm/plasma exchange

Albumin - 16.5 gm/liter exchanged
or
80 gm/plasma exchange

DISCUSSION

Recognition of the necessity for vigorous fluid resuscitation has resulted in a great decrease in the early mortality of patients sustaining major thermal injuries.[1,2,12] A number of different resuscitation regimens currently in use appear to be effective. Their key ingredient appears to be the sodium ion.[2,13] Colloid solutions offer no advantage over crystalloid solutions in the initial post-burn period, and various resuscitation fluids appear to be effective in direct proportion to their sodium content.[9,13]

Evidence suggests that despite their clinical success, these fluid resuscitation regimens have little effect on the pathophysiologic mechanisms of burn shock, and serve chiefly to maintain extracellular (ECF) volume until capillary integrity and cardiovascular function recover spontaneously at 24-36 hours post-burn.[2] Much additional work has endeavored to elucidate the optimal fluid therapy for burn patients. Isotope dilution studies have demonstrated the loss of up to 50% of the ECF volume during unresuscitated burn shock.[8] This is accompanied by an immediate decrease in cardiac output, increased pulmonary and systemic vascular resistance, increased capillary permeability, and other cardiovascular effects that do not appear to depend primarily on intravascular volume. Effective fluid resuscitation does not largely affect the initial depression of cardiac output and altered microvascular permeability, which are characteristic of burn shock.[8] At the cellular level, burn shock is accompanied by disturbances in the membrane potential, loss of normal ion homeostasis, and depressed function of energy-dependent transport mechanisms.[14-17] These effects also persist despite fluid resuscitation, which appears clinically to salvage the patient from shock.

The volume of isotonic crystalloid fluid necessary to maintain ECF volume and cardiovascular function during burn shock appears to be in the range of 2-4 cc/kg/% TBSA burn in the first 24 hours post-burn.[8,13] The clinical success attributed to varying resuscitation regimens raises doubt as to the physiologic exactitude and therapeutic specificity of any one formula, and speaks more for the physiologic reserve and compensatory mechanisms of the majority of burn patients.[3] However, these regimens have proven unsatisfactory in certain high-risk patient groups, including patients at the

extreme of age, patients with massive tissue trauma (including electrical and fourth-degree injury) or associated injury (most notably inhalation injury), patients in whom there is a significant delay in the initiation of fluid therapy, and patients in whom the presence of pre-existing illness limits cardiovascular and metabolic reserve. These patients tolerate poorly the inevitable period of hypovolemia and cardiovascular instability. Many fail to recover with fluid resuscitation alone, and develop a progression of vascular leakage and cardiac dysfunction until death.[1,2] In addition, many patients who are adequately resuscitated suffer morbidity as a result of the fluid infusion and edema that accompany resuscitation.

An expanding body of evidence supports the concept that the etiology of burn shock may be secondary to a number of alterations in the internal environment of the organism. Baxter et al., in 1955, demonstrated in cross-perfusion studies from burned to unburned dogs a decrease in cardiac output in the unburned animals, suggesting that there were circulating factors in the burn serum responsible for burn shock.[4] A number of other studies have addressed the issue of a circulating "myocardial depressant factor" or "shock toxin" responsible for the characteristic dysfunction of myocardial contractility observed in burn shock both in experimental animals and in man.

A variety of circulating serum factors have been implicated in the pathophysiology of burn shock, including the release and consumption of many mediators of the inflammatory response, which lead to increased vascular permeability and cellular membrane dysfunction.[18-20] These substances include vasoactive amines, products of platelet and complement activation, products of arachidonic acid metabolism, kinin polypeptides, endotoxin, metabolic hormones, fibronectin, and other substances (Table 4).

TABLE 4. PRESUMED CIRCULATING MEDIATORS OF BURN SHOCK

Vasoactive amines: histamine, serotonin
Products of platelet activation: thromboxanes
Products of complement activation: C_3a, C_5a, (anaphylatoxins)
Products of arachidonic acid metabolism: prostaglandins, leukotrienes
Kinin polypeptides, coagulation/fibrinolytic proteins
Exogenous substances: endotoxin
Metabolic hormones: catecholamines, cortisol
Other: neotrophil products, denatured protein, serum proteins, fibronectin

Other studies have demonstrated profound immunologic effects of sera or plasma isolated from burned patients, including enhanced red blood cell destruction, altered leukocyte chemotaxis, depressed neutrophil bacteriocidal capacity, and altered lymphocyte function.[21-26] Although specific etiologic factors are yet to be identified, the burn literature is replete with reports demonstrating that specific cellular abnormalities can be returned toward normal when the particular cell is removed from the burn environment (i.e., fresh frozen plasma or donor whole blood).[19-26] In addition to the activation and release of postulated toxic effector substances, thermal injury is associated with the consumption of a number of serum components, including fibronectin, complement components, platelets, and other inflammatory mediators.[18,25,27]

With the development of *in vivo* blood cell separators, the separation and removal of specific blood components for therapeutic intent have become possible. Plasmapheresis (selective removal of plasma) has greatly aided the management of a number of medical conditions, including antibody-mediated diseases, immune complex disorders, diseases of excess plasma factors, and numerous miscellaneous disorders.[28,29] The success of plasmapheresis in these disorders has provided direct evidence for the involvement of circulating factors in the pathogenesis of disease. Since FFP was used to replace the plasma removed by plasmapheresis, plasma exchange is a better term for the procedure employed in burn patients. The rationale for using FFP as replacement fluid is that it is relatively inexpensive, it is readily available, and it is an excellent source of coagulation factors, serum transport and binding proteins (albumin), opsonic proteins (fibronectin, complement, immunoglobulins), and other components depleted post-burn. More importantly, FFP has been shown to reverse specific leukocyte abnormalities in various *in vitro* systems.[19,22,23,26] Plasma exchange has been used successfully as a therapeutic modality to remove circulating complexes and/or to replace specific deficiencies in the serum in a variety of clinical settings.[28,29]

These concepts have led to the use of plasma exchange in an attempt to remove circulating toxic factors, replenish injury-triggered host deficiencies, and correct cellular membrane defects in burn shock. No

previous investigations utilizing this approach have been reported in burn patients. A brief report by Baxter utilizing exchange transfusion therapy in pediatric burn patients not responding to conventional resuscitation showed a reversal of fluid requirements toward normal.[1,30] Experience with the use of plasma exchange in the management of shock is limited. Levinson and Hume demonstrated improved clinical parameters following whole blood exchange in septic shock.[31] The application of intensive plasma exchange in crisis situations has demonstrated its efficacy as a therapeutic measure to allow the patient to "tide over" a potentially life-threatening or end-organ-damaging phase of a disorder when conventional therapy fails to control the disease process.

It is clear from the above discussion that an altered internal environment exists following major thermal injury. Plasma exchange may provide a potential alternative in burn shock resuscitation by returning the altered internal milieu toward normal. Whether the mechanism of action of plasma exchange involves a removal of circulating toxic factors, or a replenishment of specific deficiencies, or a combination of the two, remains to be determined. Although the plasma exchange procedure resulted in a positive albumin balance, the net gain in albumin of only 80 grams per plasma exchange procedure suggests that the positive effect of plasma exchange is not due merely to colloid replacement. Although this report is preliminary and involves a heterogeneous patient population without adequate controls, it is encouraging that the various clinical parameters return toward normal in nearly all of these patients. Clearly, a randomized trial of plasma exchange in the treatment of burn shock is indicated. Nonetheless, we believe that the following conclusions are warranted from this study: (1) plasma exchange can be performed safely in critically injured burn patients; (2) plasma exchange arrests ongoing burn shock through an as-yet-undefined mechanism, resulting in dramatic reversal of fluid requirements, brisk diuresis, resolution of lactic acidosis, and restoration of capillary integrity; and (3) plasma exchange facilitates resuscitation from burn shock in a select group of patients who do not respond to conventional volume therapy.

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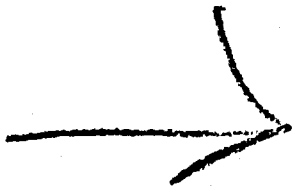
DISCUSSION PERIOD WITH DR. WARDEN

DR. METT: Did your plasma exchange alter the edema that occurs? Also, did any patients have their resuscitation started in the first 30-60 minutes?

DR. WARDEN: I will answer the second question first. Some of the patients did have it started early, but those patients usually had severe inhalation injuries, very large burns or electrical injuries. As far as edema formation, it is generally related to the amount of fluid that is given. These patients were in resuscitation failure so they all had massive edemas and required six cc's per kilogram per percent burn. We have now initiated a prospective study looking at normal burns and from the initial group it looks like plasma exchange will probably cut down the resuscitation fluid to around 1 cc per kilogram per percent burn in a "normal burn patient."

SEPSIS

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MIXED INFECTIONS AND THEIR CONTROL

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INTRODUCTION

Massive trauma predisposes the patient to bacterial invasion and sepsis as a consequence of the catabolic influence of hypermetabolism and resulting immune deficiency.[1] Systemic infection is a common complication of multiple injury despite the availability of potent and specific antibiotics.[2]

Infections following trauma are due to opportunistic pathogens that originate from endogenous or exogenous sources. These pathogens, often present as mixed infections, depend on the body site traumatized, the nature and severity of the trauma, and the circumstances of the injury. These organisms are often of enteric origin, and include Pseudomonas aeruginosa, Staphylococcus aureus, Proteus sp., Escherichia coli, Klebsiella pneumoniae, Clostridium sp., and Candida albicans. Recent work has shown that anaerobic organisms can also participate in the infectious process.[3] The anaerobes most frequently recovered are anaerobic Gram-positive cocci and the Bacteroides fragilis group. Colonization patterns established by opportunistic pathogens are dynamic, and flora found in wounds shortly after admission may not be the same as those found several days later.

Whole-body irradiation is associated with fatal septicemia in animals.[4] Postirradiation infections can also occur in man. Lymphatic and other tissues from Japanaese patients dying from the effects of the atomic blasts at Hiroshima and Nagasaki frequently revealed microscopic bacterial colonies of both Gram-positive and Gram-negative bacteria in the tissues.[5] In some cases of accidental whole-body exposures, infection with enteric organisms also occurred and presumably added to the radiation syndrome.[4] When a combination of trauma and other injuries occur in conjuction with irradiation, the risk of developing a serious infection is increased. Following such combined injury,

the role of proper therapy with antimicrobial agents is of primary importance. Studies have shown that appropriate management of these infections can reduce the morbidity and mortality following combined injury.[6]

A factor that complicates management of trauma-induced infections is that most of them are polymicrobial, including multiple aerobic and anaerobic organisms. Furthermore, due to the depletion of the host immune defenses, bactericidal antibiotics are preferred, and synergistic combinations of agents producing bactericidal action should be used.

The complex microflora associated with pyogenic wound and soft tissue infections generally reflect the indigenous flora of the skin or adjacent mucous membranes of the oropharyngeal, gastrointestinal, or genital tracts. Necrotizing wound and soft tissue infections are particularly prone to develop in areas with tissue ischemia and lowered oxidation-reduction potential. Risk of infection is also great at anatomic sites regularly exposed to fecal or oral contamination.

WOUND AND SKIN INFECTIONS

Beta-hemolytic streptococcus and S. aureus, either alone or in combination, are usually the causative organisms in skin infections.[7] Wounds associated with foreign bodies can be infected with P. aeruginosa.[7] Also many wound and skin infections following trauma are caused by mixed flora that are endogenous in nature and act synergistically.

Crepitant cellulitis is an acute anaerobic infection of the soft tissue that is characterized by abundant connective tissue gas and minimal systemic toxicity. Clostridium perfringens or other clostridial specimens are generally present in these lesions.[8] Other organisms that can be involved are Bacteroides, Peptostreptococcus, and coliforms.[9] Necrotizing fascitis, a gangrenous lesion, is generally caused by a variety of organisms including beta-hemolytic streptococci, S. aureus, Gram-negative enteric organisms, Peptostreptococcus, Bacteroides, and Fusobacterium.[10] Gas gangrene is a rapidly progressive, life-threatening, toxemia due to Clostridium infection of

muscle. It usually follows contamination of severe crushing muscle injury by animal or human feces.[1] C. perfringens or other clostridial species are isolated from most of the cases, sometimes mixed with other anaerobes and facultative organisms. Synergistic necrotizing gangrene is caused by the combination of (a) a microaerophilic nonhemolytic streptococcus, found primarily in the spreading periphery of the lesion, and (b) S. aureus in the zone of gangrene. A variety of other organisms can be seen instead of or in addition to the staphylococci. These include Proteus, Enterobacter, Pseudomonas, and Clostridium species.[11] Synergistic necrotizing cellulitis is caused by mixed infection containing one or more species of Gram-negative aerobic bacteria and at least one obligate anaerobe such as Bacteroides, Peptostreptococcus, or Peptococcus.[11]

Cutaneous abscesses are commonly encountered following wound infection, and can be caused by many aerobic and anaerobic pathogens. Although their treatment is usually surgical, knowledge of the usual flora causing infection in certain anatomic loci should permit the institution of therapy before the results of cultures are available. Anaerobes predominate in abscesses in the vulvo-vaginal, buttocks, perirectal, finger, and head areas, but aerobes are more prevalent in the neck, hand, leg, and trunk areas.[7] The major aerobes recovered are S. aureus, group A beta-hemolytic streptococci, Enterobacter, and E. coli. The common anaerobes recovered include anaerobic Gram-positive cocci, Bacteroides sp., and Fusobacterium sp.[7]

INFECTIONS FOLLOWING BLUNT TRAUMA

Microbial infection in impact and crushing injuries is of secondary importance to the original injury. In severe trauma, there may be multiple injuries to the head, chest, and abdomen as well as fractures of the extremities and crush injuries. The first concern is survival of the patient and maintaining vital functions. Frequently, severe injury is associated with impairment of host defense mechanisms, and the stage is set for subsequent serious infection. The two possible sources of microbial contamination at this time are the host and the environment.

The first and most common method of developing infection secondary to blunt trauma is a break in the mucosal barrier, which gives bacteria ready access to the peritoneal or pleural cavities. Bacteria from the patient's gastrointestinal and respiratory tracts may find egress from lacerations or disruptions of either tract. Rupture of any hollow viscus in the abdomen is followed by bacterial seeding of the peritoneal cavity.

Bacteria can also enter the tissues of the host and cause infection by secondary invasion.[8] A large hematoma, hemothorax, or any area of impaired blood supply is a favorable medium for the growth of endogenous microorganisms. Exogenous bacteria are usually not prime pathogens, and cause disease only if the local wound is not properly treated.[8]

INFECTIONS FOLLOWING PENETRATING INJURIES

Penetrating injuries occur in any part of the body. They are caused by a variety of agents, ranging from high-velocity bullets and shrapnel to knives and splinters. Many kinds of microorganisms cause infection following a penetrating injury. What is carried into the wound by the penetrating agent is important, as is the location of the wound and the organs that are perforated. Although almost any combination may occur, microorganisms from the gastrointestinal and respiratory tracts predominate.[7,8]

The wounding agent inevitably causes tissue destruction, usually introduces some foreign matter, and is associated with some degree of bleeding in the tract of penetration. This process establishes a culture medium suitable for microbial replication. With or without foreign matter, necrotic tissues and hematomas provide ideal conditions for growth: protection from phagocytes and humoral antibodies, depletion of oxygen and enhanced growth of microaerophilic and anaerobic microorganisms. When the penetrating wound enters the gastrointestinal tract, urinary tract, or respiratory tract, there is the serious complication of contamination by microorganisms resident in the host.

INTRAABDOMINAL INJURY

Secondary peritonitis and intraabdominal abscesses can be due to penetrating wounds. The infection is due to the entry of enteric microorganisms into the peritoneal cavity through a defect in the wall of the intestines or other viscus. The peritonitis following the rupture of a viscus is usually a synergistic infection. The specific microorganisms involved in peritonitis are generally those of the normal flora of the gastrointestinal tract where anaerobic bacteria outnumber aerobes in the ratio 1:1,000.[12] The presence of mixed aerobic and anaerobic flora in the peritoneal cavity was demonstrated in patients with ruptured viscus,[13] and these organisms were also recovered from the postoperative wound.[14]

Peritonitis is an excellent example of a synergistic infection between aerobic and anaerobic microorganisms. The two types of bacteria have opposite oxygen requirements, and the alteration that each causes in its environment as it grows permits the rapid proliferation of their partners.[10] The principal anaerobic pathogens are B. fragilis, Clostridium sp., and anaerobic Gram-positive cocci. Coliforms and facultative streptococci were frequent cohabitors.

BURN INFECTIONS

The most serious and common complication of burns is infection. A third-degree burn is more likely to be associated with severe infection than is a partial-thickness burn. Infection may be localized to the site of the burn or may be manifested as an overwhelming general sepsis. Burn wound sepsis is a major cause of death among burn patients.[15] Sepsis is characterized by progressive bacterial proliferation within the burned tissue, invasion into adjacent tissue, and systemic dissemination.[16]

Microorganisms usually gain access to burns directly from the skin. Soon after a burn injury, surface cultures may reveal multiple organisms. Within 3 to 5 days, the wound will become colonized by one or two specific organisms that have survived the competition with other microorganisms, or have proven

particularly resistant to burn wound therapy. The burn victim's diminished humoral and cellular defense systems make him more susceptible to infection. Deficiencies in the inflammatory response include diminished chemotaxis; diminished ability of the neutrophils to phagocytose and thereby kill offending bacteria; and a decrease in opsonin and antibody, which renders the bacteria susceptible to phagocytosis.

Streptococci were the principal burn pathogens in the past; currently S. aureus is much more commonly encountered. Gram-negative bacilli, especially P. aeruginosa, and fungi are also detected as the predominant pathogens in burn wounds. Anaerobes belonging to the Bacteroides and Fusobacterium sp. can be found in burns in the oral and anal areas.[17]

INFECTIONS FOLLOWING IRRADIATION

The severe hematological and gastrointestinal injury caused by irradiation makes the affected individual more susceptible to exogenous infections and to septicemia due to spread of his own indigenous flora. Most of the data in this field were obtained from studies done in animal models. However, much can be learned from the susceptibility to infections of individuals immunosuppressed by other means.

The predominant organisms causing sepsis following irradiation are E. coli, Proteus sp., P. aeruginosa, Enterococci, and S. aureus.[4] Anaerobic bacteria such as anaerobic Gram-positive cocci and B. fragilis are also recovered from irradiated animals.[3] The infections that develop in irradiated animals are generally polymicrobial due to mixed aerobic and/or anaerobic bacteria.[3,4]

BACTERIAL SYNERGISM

Polymicrobic infections are more pathogenic for experimental animals than are those involving single organisms.[18] The potential importance of synergy such as this was first emphasized by Altemeier who noted a direct correlation between peritonitis mortality rates and the number of bacterial species

cultivated from the peritoneal fluid.[19] Support for this thesis was provided by showing that intraperitoneal challenge with the isolates in pure culture was generally well tolerated by animals, but combinations of the various isolates produced rapid lethality.[20] A similar observation was noted by Meleney, who studied synergism between E. coli, C. perfringens, and a nonhemolytic streptococcus.[21]

McDonald et al. studied synergistic interaction between aerobes and anaerobes, and found that B. melaninogenicus was indispensable in producing abscesses following subcutaneous injections into animals.[22] However, it was necessary to include another microbe in the inoculum to provide a source of vitamin K_1, which is a growth requirement for B. melaninogenicus.[23] A similar mechanism of synergy is seen with foot rot in sheep, in which Fusobacterium necrophorum is the invading microbe, but its required growth factors are supplied by the concurrent presence of Corynebacterium.[24] This synergistic interaction is somewhat more complicated because F. necrophorum also protects its nutrient supply with the production of a leukocidin that prevents phagocytosis of the Corynebacterium.

Another mechanism of synergy was described by Meleney in his classical studies of synergistic bacterial gangrene.[25] He found that cultures from the central bed of the ulcer yielded S. aureus and a microaerophilic streptococcus, but cultures from the advancing edge of inflammation showed only the latter organism. This lesion could be reproduced in experimental animals only with an inoculum composed of both bacteria. Subsequent work indicated that the role of the S. aureus was to produce hyaluronidase, which promoted the invasive potential of the microaerophilic streptococcus.[26]

In recent studies we have demonstrated the ability of "helper" organisms, generally recovered mixed with anaerobes, to induce capsule formation in unencapsulated Bacteroides sp.[27] These Bacteroides sp. included strains of B. melaninogenicus and fragilis groups, B. oralis, and B. ruminocola ssp. brevis. The previously non-encapsulated Bacteroides species were non-pathogenic in vivo, and did not cause abscesses following their inoculation into animals. However, following their co-inoculation with abscess-forming organisms, they

acquired capsular material, and were thereafter able to cause abscesses by themselves. This phenomenon can be due to various yet-undetermined mechanisms. One could be due to *in vivo* transfer to DNA from encapsulated to unencapsultated organisms. An alternate explanation is that the presence of capsular material from the "helper" organism was sufficient to prevent phagocytosis of the organisms and permit the selection of encapsulated organisms. It is postulated that a selection process was the mechanism responsible for the phenomenon, due to the presence of a few encapsulated organisms in populations of the initially non-encapsulated strains. Selection *in vivo* of encapsulated *Bacteroides* sp., with the assistance of other encapsulated, or non-encapsulated but abscess-forming aerobic and anaerobic organisms, may explain the apparent conversion into pathogens of non-pathogenic organisms that are part of normal host flora. This phenomenon could contribute to the ability of *B. fragilis* (which constitutes only about 0.5% of the normal fecal flora) to become a pathogen present in 70% to 80% of intra-abdominal infections.

In other studies (unpublished data), we found synergy between anaerobic Gram-positive cocci and *Bacteroides* sp. or *Pseudomonas aeruginosa*. The number of bacteria required to cause lethality or abscess formation was reduced by 15-fold or more when a combination of microbes was used rather than single strains alone.

The experimental data presented demonstrate the important role of facultative bacteria in mixed aerobic and anaerobic infection. The mechanisms of their influence on the infectious process may include the promotion of an appropriate environment for anaerobic growth, the production of necessary nutrients, the production of extracellular enzymes to promote tissue invasion by the anaerobe, and assistance in selection of encapsulated strains.

MANAGEMENT OF INFECTIONS FOLLOWING TRAUMA AND IRRADIATION

The strategy for therapy of post-trauma infections includes surgical drainage of pus, debridement of any necrotic tissue, and appropriate use of antibiotics. Certain types of adjunctive therapy, such as hyperbaric oxygen, may also be useful.

Surgery may be the only therapy required in some cases, such as localized abscesses or decubitus ulcers without signs of systemic involvement. However, antibiotics are indicated in the majority of patients whenever systemic manifestations of infection are present or when suppuration either extends or threatens to spread into surrounding tissue. In many infections, antimicrobial therapy alone is sufficient; in others, it is an important adjunct to the surgical approach.

Selection of antimicrobial agents is simplified when results of culture from a reliable specimen are available. This is seldom the case, however, in infections involving anaerobes, and many patients are treated empirically on the basis of suspected rather than established pathogens. Fortunately, the types of bacteria involved in many infections and their antimicrobial susceptibility patterns tend to be predictable. However, some bacteria have become resistant to antimicrobial agents, and many can become resistant while a patient is receiving therapy.[28] Other factors may also influence the choice of antimicrobial therapy, e.g., pharmacologic characteristics of the various drugs, their toxicity, their effect on the normal flora, and bactericidal activity.[8]

ANTIMICROBIAL AGENTS

Since anaerobic bacteria mixed with aerobic organisms are generally recovered in many infections, the selection of proper therapy may become complicated. The choice of the appropriate antimicrobial agents, therefore, should provide adequate coverage for most of the pathogens recovered. Table 1 summarizes the antimicrobial agents effective against most organisms present in mixed infections.

PENICILLIN. This antibiotic is effective against aerobic streptococci and most anaerobic species except those that produce beta-lactamase, which are generally susceptible to penicillin. *B. fragilis* is resistant to penicillin[28] resistance to penicillin is also appearing in growing numbers of other *Bacteroides* species (e.g., *B. melaninogenicus* and *B. oralis*) as well as strains of *Clostridium*, *Fusobacterium*, and microaerophilic streptococci.

Methicillin, nafcillin, and the isoxazolyl penicillins (oxacillin, cloxacillin, and dicloxacillin) have excellent activity against S. aureus but have unpredictable activity against anaerobes and are frequently inferior to penicillin G.[8]

Antimicrobial Agent	Anaerobic bacteria		Aerobic bacteria	
	B. fragilis gr.	Other anaerobes	Gram-positive cocci	Enteric
Penicillin[a]	poor	excellent	good	poor
Chloramphenicol[a]	excellent	excellent	good	variable
Cepholothin*	poor	good	good	poor
Cefoxitin[b]*	excellent	excellent	good	good
Moxalactam[c]	excellent	excellent	good	good
Clindamicin*	excellent	excellent	very good	poor
Carbenicillin-ticarcillin[d]	good	excellent	good	very good
Metronidazole[e]	excellent	excellent	poor	poor

* = does not penetrate the central nervous system

[a] Poor for S. aureus

[b] Not effective against P. aeruginosa, Enterobacter sp., S. faecalis

[c] Not effective against enterococci, some strains of B. fragilis, P. aeruginosa

[d] Some centers have reported increased resistance; no activity against S. aureus or K. pneumoniae.

[e] Anaerobic Gram-positive bacilli may be resistant.

CARBENICILLIN AND TICARCILLIN. These penicillin derivatives have good in vitro activity against most strains of B. fragilis as well as other penicillin-sensitive anaerobes[28] and P. aeruginosa.

CEPHALOSPORINS. The antimicrobial spectrum of first - generation cephalosporins against anaerobes is similar to that of penicillin G, although they are less active per unit weight. Most strains of B. fragilis and many of B. melaninogenicus are resistant by virtue of cephalosporinase production[8,28]. Cefoxitin, a second-generation cephalosporin, is relatively resistant to this enzyme and is therefore effective against B. fragilis. The third-generation cephalosporins have a broad spectrum of activity against enteric Gram-negative bacilli and most strains of B. fragilis.[28]

CHLORAMPHENICOL. This drug is very active against anaerobes and many Gram-negative enteric organisms.[8,28] It is the drug of choice for treatment of anaerobic infections of the central nervous system.

CLINDAMYCIN. Clindamycin has a broad range of activity against anaerobic organisms, including B. fragilis, and is effective against S. aureus and streptococci. The primary manifestation of toxicity with clindamycin is colitis. It should be kept in mind that colitis has been associated with a number of other antimicrobial agents, such as ampicillin and many cephalosporins.

METRONIDAZOLE. This antibiotic has excellent in vitro activity against most obligate anaerobes, including B. fragilis.[28] Aerobic and faculative anaerobes, such as coliforms, are usually highly resistant.

AMINOGLYCOSIDES. This group of agents (gentamicin, amikacin tobramicin) are very effective against Gram-negative enteric aerobic bacteria, and they possess some activity against S. aureus. However, they are inactive against anaerobic bacteria. They manifest synergistic activity with penicillins against S. aureus, Group B streptococci, Listeria monocytogenes,[29] and B. melaninogenicus.[30]

CLAVULANIC ACID. Clavulanic acid is a beta-lactamase inhibitor that resembles the nucleus of penicillin. It irreversibly inhibits beta-lactamase enzymes produced by some enterobacteriaceae, staphylococci,[31] and Bacteroides species.[32] Clavulanic acid and other beta-lactamase inhibitors have very weak

antibacterial activity alone, but when used in conjunction with a beta-lactam antibiotic, they are effective in treating infections caused by beta lactamase-producing bacteria. Its usefulness in the chemotherapy of human infections is currently being evaluated.

SYNERGISTIC ANTIMICROBIAL COMBINATIONS

Combinations of antibiotics are continually being studied in attempts to discover more effective therapy for serious infections. Combined therapy might delay the emergence of antimicrobial resistance, provide broad-spectrum coverage for infections of unknown or mixed etiology, or generate a greater antibacterial effect against specific pathogens than is achievable with a single drug. The improved killing of the offending anaerobic organisms, as expressed by effective bactericidal activity, is especially important in the treatment of endocarditis, bacteremia, and closed-space infections, such as brain or lung abscesses that cannot be surgically drained.

Combination therapy should not be used indiscriminately, for two reasons. First, the risks of adverse reactions are increased when multiple drugs are administered. Second, combination therapy is sometimes less effective than a single drug against a specific pathogen.[29]

Synergistic interaction between aminoglycosides and penicillins against aerobic organisms has been observed. This combination is effective in the treatment of enterococcal and staphylococcal diseases. It is postulated that the penicillin, which inhibits bacterial cell wall synthesis, enhances the penetration of aminoglycosides, which have a lethal effect on the ribosomes. B. fragilis, a strict anaerobe, is resistant to aminoglycosides, because these agents are poorly transported into facultatively anaerobic bacteria under anaerobic conditions.[33] However, a recent study[34] demonstrated that the ribosomes of the strictly anaerobic bacteria C. perfringens and B. fragilis are susceptible to the action of streptomycin and gentamicin. The susceptibility of the Bacteroides ribosome to aminoglycosides, combined with the ability of penicillin to alter the organisms' membranes, suggests a possible explanation for the recently observed synergistic combination between the agents against B. melaninogenicus.[29]

BETA-LACTAMASE PRODUCTION

Many aerobic and anaerobic microorganisms, including B. fragilis, produce beta-lactamase, which enables them to resist penicillin.[35] Until recently, most B. melaninogenicus and B. oralis strains were considered susceptible to penicillin. However, within the past decade, penicillin-resistant strains have been reported with increasing frequency.[35]

The appearance of pencillin resistance among Bacteroides sp. has important implications for chemotherapy. These organisms may release beta-lactamase into the environment, thus degrading penicillin and protecting not only themselves but also other penicillin-sensitive pathogens. Therefore, penicillin therapy directed against a susceptible pathogen might be rendered ineffective by the presence of a penicillinase-producing organism.

Several studies demonstrate the activity of this enzyme in clinical infections. Louvois and Hurley demonstrated the degradation of penicillin, ampicillin, and cephaloridine by purulent exudates obtained from four of 22 patients with abscesses.[36] Beta-lactamase activity has also been found in empyema fluid[37] and in samples of pus obtained from 12 patients with polymicrobial intra-abdominal abscesses or polymicrobial empyema.[38]

The importance of beta-lactamase production in anaerobic infections was demonstrated by Hackman and Wilkins,[39] who were able to show that penicillin-resistant strains of B. fragilis, B. menaninogenicus, and B. oralis could protect F. necrophorum from penicillin therapy in mice. O'Keefe et al.[40] demonstrated inactivation of penicillin-G in an experimental B. fragilis infection model in the rabbit peritoneum.

We have recently demonstrated the ability of beta lactamase-producing B. fragilis and B. melaninogenicus to protect group A beta-hemolytic streptococci from penicillin in mice.[41] We also observed that the beta-lactamase produced by aerobic organisms (such as K. pneumoniae or S. aureus) had a protective effect on penicillin-susceptible B. melaninogenicus. Penicillin was ineffective

in eradicating the penicillin-susceptible anaerobe in the presence of the aerobic beta-lactamase producer; however, the combination of clavulanic acid and pencillin was effective.

The results of all of these studies raise questions concerning the efficacy of beta-lactamase-susceptible antibiotics against beta-lactamase-producing aerobic and anaerobic bacteria. In seriously ill patients with mixed infections where beta-lactamase-producing bacteria are present, administering antibiotics that are effective against these beta-lactamase producers should be considered. The recent development of potent enzyme inhibitors, such as clavulanic acid, may facilitate a new approach to this problem.

IMPORTANCE OF THERAPY OF ALL COMPONENTS OF MIXED INFECTION

The necessity for treating all components of mixed infections has now been adequately documented in both experimental and clinical studies. The importance of synergistic antimicrobial therapy that will be effective against both aerobic and anaerobic bacteria present in a mixed infection was demonstrated in animal models for treatment of intra-abdominal infection. Peritonitis was induced in rats by introducing gelatin capsules containing cecal contents into their abdominal cavities.[42] The animals that survived the initial septicemic stage caused by coliforms developed intra-abdominal abscesses caused by anaerobes. An evaluation of the effect of therapy with clindamycin, gentamicin, or a combination of both was done. It was shown that the untreated control group and the clindamycin-treated group had identical mortality rates of about 35% due to *E. coli* sepsis. However, administration of gentamicin alone or in combination with clindamycin led to greater than 90% survival. The data suggest that the early mortality in the peritonitis and septicemic phase is attributable to gentamicin-sensitive coliform bacteria. The effect of this treatment on abscess formation was entirely different. All untreated animals that survived developed abscesses due to *B. fragilis*, as did those treated with gentamicin alone. However, the use of clindamycin alone or in combination with gentamicin was associated with a greatly reduced incidence of abscesses from 100% to only 5%. These findings

indicate that anaerobes may be responsible for complications following abdominal perforation, such as intra-abdominal abscess formation, and show that optimal treatment of intestinal perforation requires a drug to control both aerobic and anaerobic bacteria.

Clinical work also supports these animal data. Thadepalli et al.[43] treated 100 patients with a perforated small or large intestine. Two regimens were used. Fifty-two patients received a cephalosporin-kanamycin combination and 48 received clindamycin plus kanamycin. Since both groups were provided with kanamycin activity against coliforms, the point of comparison was between cephalothin (poor antianaerobic activity) versus clindamycin (excellent antianaerobic activity). In the cephalothin group, 14 patients developed abscess, wound infection, or septicemia, compared with only 5 patients in the clindamycin group. Anaerobes, moreover, were involved in 11 episodes of septic complications in the patients receiving cephalothin but in only 1 episode in those receiving clindamycin. Many other studies have shown similar results. These studies demonstrate the need for directing therapy at the anaerobic component of mixed infections, in addition to the aerobic component, for optimal therapeutic results.

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DISCUSSION PERIOD WITH DR. BROOK

DR. VAN DER WAAIJ: Do you have an explanation for the transfer of the genetic information from perhaps the E. coli to the B. fragilis concerning the formation of capsule? Was it capsule conjugation or transformation?

DR. BROOK: We believe that it is a selection process. However, we don't have a complete explanation. We did find that some organisms among the groups that we called the non-encapsulated, in the early stages, before we injected them for the first time in mice, did have a capsule. There probably was a selection process for encapsulated bacteria in the animal.

If we did interrupt the experiment within less than 7 to 10 days, we couldn't find many encapsulated organisms. So it was not a phenomenon of all or none; it was a selection for a population that was encapsulated from the beginning.

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SEPTIC SHOCK: PERSISTING UNCERTAINTIES RELATING TO PATHOPHYSIOLOGIC MECHANISMS AND THEIR MEDIATORS

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INTRODUCTION

In combined injuries, as with all other types of severe trauma, sepsis will undoubtedly prove to be the major cause of fatalities among those patients who have survived initial resuscitation. Improved techniques in the management of major trauma have led to substantial reduction in certain types of post-traumatic sepsis. The almost unbelievably high rates of gas gangrene observed during World War I casualties were so effectively controlled by adequate extensive debridement, cleaning of wounds, and other measures that this hideous complication became much less frequent during susbequent conflagrations. Experiences in Vietnam and the Israeli-Arab conflicts, however, demonstrated that other types of sepsis still remain one of the primary causes of death for persons who would otherwise survive their injuries. It is almost certain that the immunosuppressive effects of irradiation, coupled with major traumatic injuries, will result in infections of a magnitude and rate surpassing any previously observed. This potential situation might best be appreciated by imagining a combination of the infectious problems seen in patients with extensive thermal injuries combined with those observed in patients with leukemia or other neoplasms receiving intensive chemotherapy.

Gram-negative bacilli have become the predominant etiologic agents of post-traumatic and nosocomial infections over the past 3 decades, and there is no evidence that the prevalence of these infections is abating.[1,2] Experiment indicates that the continuing introduction of new antimicrobials or attempts at various control measures has not had a major impact on the prevalence or the excessive fatality rates of such infections. In actuality, newly introduced antibiotics have only extended the spectrum of activity to Gram-negative bacilli resistant to currently available antibiotics rather than

providing enhanced effectiveness against susceptible organisms.[3] This suggests that introduction of additional new agents will not have a major impact on Gram-negative bacillary infections and that improved therapy is dependent on new approaches such as immunotherapy as studied by us[4,5] and Dr. Braude's group,[6,7] (described by Dr. Braude in a subsequent presentation), and the development of more effective management of complications that are important contributors to the outcome of such infections.

SHOCK

Although shock may accompany a variety of infections, it appears to be uniquely associated with bacteremia caused by Gram-negative bacilli with regard to both its frequency and its importance as a crucial determinant of lethality. Clinical studies of ours of approximately 800 patients with Gram-negative bacteremia and those from other centers have indicated that shock occurs in approximately 40 percent of patients and that the species of etiologic agent, including both aerobes and anaerobes, does not influence its frequency.[8,9] The importance of shock as a determinant of lethality in Gram-negative bacteremia is illustrated in Table 1. Since earlier studies have demonstrated that the severity of the host's underlying disease is a major determinant of the outcome of bacteremia,[8,9] comparisons of fatality rates in bacteremia with and without shock are made among groups of patients stratified by severity of underlying host disease in that Table. Bacteremia complicated by the development of shock was several-fold more lethal than bacteremia not complicated by shock in each category of underlying host disease and, overall, bacteremic shock was associated with a seven-fold increase in fatality over that of bacteremia without shock. These and other observations clearly document the excess mortality when bacteremia is complicated by the development of shock. Other studies have demonstrated that early appropriate antibiotic therapy both decreases the frequency of development of shock and improves survival after its occurrence.[9] They have also indicated that passively administered antibody to some shared antigens of Gram-negative bacilli improve survival,[7] but the fatality rates in bacteremic shock still remain unacceptably high.[10] The continued high fatality rates of shock in Gram-negative bacteremia clearly emphasize the necessity for more complete

delineation of the basic mechanisms involved in the pathogenesis of bacteremic shock as a necessary precursor to the development of more effective therapeutic measures.

TABLE 1. FATALITY RATES IN GRAM-NEGATIVE BACTEREMIA WITH AND WITHOUT SHOCK

Underlying Host Disease	Fatality Rates
Rapidly Fatal	
Shock	14/22 (64%)*
No Shock	3/21 (14%)
Ultimately Fatal	
Shock	79/150 (52%)
No Shock	17/157 (11%)
Non-fatal	
Shock	34/97 (35%)
No Shock	5/165 (3%)
All	
Shock	127/269 (47%)
No Shock	25/343 (7%)

* Number of fatalities/number with bacteremia (percent fatal)

Despite the clinical importance of shock associated with Gram-negative bacteremia in man and the extensive investigation, serious defects still exist in our understanding of basic pathophysiologic mechanisms involved in its development. This partially reflects the tendency to assume similar pathophysiologic alterations in shock associated with infections at various sites and caused by different etiologic agents. This tendency becomes readily apparent when one observes the almost constant use of terms such as "septic shock" of "endotoxic shock" to describe any patient with hypotension and fever or, less frequently, hypothermia, irrespective of their causes. It is clear that a variety of pathophysiologic changes (listed in Table 2) are involved in the development of shock that accompanies different types of infections caused by various etiologic agents. Even in shock associated with bacteremia caused by different types of bacteria, significant differences in clinical features and hemodynamic alterations can be identified. Although shock may occur

during bacteremia caused by almost any bacterial species, its relatively high frequency and appearance within the first few hours after the onset of bacteremia with Gram-negative bacteria, in contrast to a lesser frequency and its occurrence as a late, almost agonal event in Gram-negative bacteremia, is typical of the two types.

TABLE 2. SHOCK ASSOCIATED WITH INFECTION ETIOLOGY AND PATHOGENESIS

PRIMARY PUMP FAILURE (CARDIOGENIC)

1. Viral enteroviruses (Coxsacki, Echo)
2. Bacterial myocarditis (diphtheria, Leptospira, SBE)
3. Parasitic Toxoplasma, Trichinella

VALVULAR INSUFFICIENCY

Acute and subacute bacterial endocarditis

FAILURE OF MYOCARDIAL FILLING

Pericardial effusion

INADEQUATE INTRAVASCULAR VOLUME

1. Extracellular fluid loss or sequestration
   a. Diarrheal disease (cholera, shigellosis, salmonellosis, toxigenic E. coli, etc.)
   b. Pancreatitis
   c. Peritonitis
2. Increased vascular permeability
   a. Rickettsial diseases (RMSF, typhus, etc.)
   b. Viral hemorrhagic fevers

FAILURE OF VENOUS RETURN

1. Gram-negative bacteremia
2. Candidemia

HYPOXEMIA

1. Pneumonia - bacterial and viral
2. Profound intravascular hemolysis (clostridial sepsis)

Early reports of hemodynamic measurements in "septic shock" also described disparate and often conflicting results. Subsequently, more careful studies demonstrated that these variable hemodynamic alterations were attributable to studies of heterogeneous patient populations. Many of the patients had underlying diseases which in themselves resulted in hemodynamic alterations, some of the bacteremias were caused by different classes of etiologic agents, and some measurements were carried out at different stages of shock. The careful prospective studies by Gunnar's group and other investigations have demonstrated distinctly different patterns of hemodynamic changes during the early and late phases of shock and between shock caused by Gram-negative and Gram-positive organisms.[11-13] These studies demonstrated that the early hemodynamic changes during shock assoicated with Gram-negative bacteremia consisted of (a) marked diminution in systemic resistance, (b) increased or, less often, decreased or normal cardiac output (inadequate to compensate for the decrease in peripheral resistance), (c) increased to normal stroke volume, and (d) hyperventilation. As shock progressed, peripheral vasoconstriction appeared, cardiac output diminished, and lactate accumulation became prominent. In contrast, only minimal and variable changes in systemic resistance and cardiac output were found early in the course of shock caused by Gram-negative organisms.[11-13] Delineation of the sequence of pathophysiologic alterations in the course of shock caused by Gram-negative bacilli and differences in the hemodynamic alterations in shock caused by Gram-positive organisms offers the possibility of developing more rational and effective clinical treatment of bacteremia shock.

"EFFECTORS" AND "MEDIATORS" OF HEMODYNAMIC CHANGES IN BACTEREMIC SHOCK

A number of recent scientific advances offer considerable potential for the development of newer, more effective modalities for the treatment of shock associated with Gram-negative bacteremia. There has been a literal explosion in the development of numerous specific antagonists, receptor blockers, or agents that inhibit the release of various vasoactive materials over the past decade. Such agents could offer considerable promise as effective therapeutic modalities in the treatment of shock. Before this approach can be successful, however, identification of the material(s) that induces the pathophysiologic

changes and those endogenous substances that mediate the crucial hemodynamic alterations in "septic" shock is an essential prerequisite for the development of specific therapy of this type. Indeed, the precise identification of crucial "effectors" and "mediators" may be more of a problem than development of specific inhibitors or antagonists. This problem is largely the result of a vast array of studies that propose such an extensive variety of potential effectors and mediators as to preclude sifting through the "chaff" to identify the few "germs" that can be unequivocably shown to be relevant to the crucial pathophysiologic changes in human bacteremia.

To this reviewer, one of the major reasons for our confusion over the role of various effectors and mediators has been the assumption that "bolus" injection of endotoxin or addition of endotoxin to "in vitro" systems faithfully reproduces the changes that occur during Gram-negative bacteremia in man. There is no question that administration of purified endotoxin to both man and experimental animals produces many of the effects seen in human Gram-negative bacteremia: fever, shock, lethality, and activation of the complement-fibrinolytic-coagulation-kinin synthesis. In addition, endotoxin induced an almost unlimited number of changes in an extensive variety of "in vitro" and "in vivo" biologic systems.[14] The fact that many of the changes induced in "in vitro" systems require concentrations of endotoxin considerably in excess of those required for lethality in the intact animal has not hindered claims of their relevance of such findings to human infections. These observations have led to the widespread belief that endotoxin shed by Gram-negative bacilli during infection is solely responsible for the clinical manifestations observed during Gram-negative bacillary infections.[15] In contrast, a number of other carefully performed studies cast doubt on the paramount importance of endotoxin, dissociated from the bacterial cell wall, as the sole effector of events in clinical infections. Doubt is cast on the crucial role of "free", circulating endotoxin in infection by studies of endotoxin effects in experimental human typhoid and tularemia,[16] investigations in a goat hemoperfusion model, the similarity of lethal Gram-negative[17] infections in mice whose susceptibility to endotoxin varied 5000-fold,[18] and lack of correlation of the presence or levels of circulating endotoxin (assayed by various techniques) with manifestations (fever, shock,

and death) in clinical infections attributed to endotoxin.[19-21] Overall, these findings suggest that any role of endotoxin in producing the manifestations of Gram-negative bacteremia is more complex than a simple quantitative response to endotoxin released from viable bacteria.

If there is uncertainty regarding the exact nature of the primary stimulus for the pathophysiologic alterations in Gram-negative bacteremia, the exact role of the extensive number of endogenous mediators that have been proposed to have important pathogenetic effects is even more obscure. Table 3 lists a number of endogenous products that have been proposed at various times as important mediators of critical pathophysiological changes in bacteremia. Among these proposed mediators, the role of endogenous pyrogen has been delineated most clearly.[22] It is produced by mononuclear phagocytes and is believed to be identical with or extremely similar to Interleukin I and Leukocyte Endogenous Mediator (LEM), in the production of fever during infection. Other biologic effects attributed to LEM are less clearly delineated in sepsis.[23] The postulated importance of most of the other significant mediators in the development of shock has primarily resulted from the demonstration of increased concentrations of the postulated mediator following injection of endotoxin rather than the documentation of changes in concentration during human infections. In addition, many of the studies of these mediators have failed to sequentially evaluate the changes in the concentration of these mediators in relation to the pathophysiologic alteration that they putatively induce. Additional difficulties arise in distinguishing (a) the changes in mediators that initiate pathogenetic mechanisms from (b) the changes that reflect the host's response to injury.

Among these postulated mediators, interrelated activation of the coagulation, fibrinolytic, complement, and kinin systems is best documented to occur in shock associated with Gram-negative bacteremia. These studies have demonstrated that such activation precedes the development of shock and that the magnitude of these changes parallels the severity of bacteremia.[24,25] This, coupled with the frequent occurrence of intravascular coagulation and

fibrinolysis as well as the similarity of the hemodynamic changes induced by bradykinin with those early in the course of Gram-negative bacteremia shock, suggests their pathophysiologic significance in clinical disease.

TABLE 3. PROPOSED MEDIATORS OF THE PATHOPHYSIOLOGIC EVENTS IN BACTEREMIA

Endogenous pyrogen (leukocyte endogenous mediator Interleukin I)
Catecholamines (epinephrine-norepinephrine)
Serotonin
Histamine
Acetylcholine
Glucocorticoids
Kinins
Complement components (anaphylotoxins)
Coagulation components
Endorphins
Prostaglandins
Lysosomal components
Slow reacting substance
Macrophage and lymphocyte products
Myocardial depressant factor

Endogenous opiates (endorphins) and various prostaglandins like compounds have become fashionable as putatively important mediators of septic shock. Administration of endotoxin has been shown to induce increased serum levels of endorphins prior to the development of shock.[26] Subsequent uncontrolled clinical studies reported improvement in blood pressure levels in patients with shock secondary to bacteremia after the administration of the opiate inhibitor naloxone.[27] Regrettably, blinded, randomized studies of approximately 40 episodes of septic shock by our group and our surgical colleagues failed to demonstrate any significant increase in blood pressure levels in patients treated with naloxone in comparison to those receiving placebo.[28] Similarly, endotoxin administration has been demonstrated to induce the release of prostaglandins or similar compounds, and the prostaglandin or thromboxane synthesis inhibitors indomethacin and ibuprofen have been shown to prevent or ameliorate endotoxin-induced hypotension.[29] Elevated levels of PGF_2 and thromboxane have also been demonstrated in patients dying of sepsis.[30] Further delineation of the role of this class of mediators is not available, and the uncertainty relating to their actual role

in the pathogenesis of bacteremic shock can be summarized by one author's statement that "Prostaglandins have been suggested as both etiologic and therapeutic agents in endotoxin shock."[31]

Thus, despite years of intensive investigation and accumulation of a vast volume of investigative information, we are left with an extensive list of potential "effectors" and "mediators" of the pathophysiologic changes in septic shock, but we are still in doubt concerning those that are of crucial importance in determining lethality and those that merely represent "epi" or "paraphenomena." The increasing availability of specific inhibitors or antagonists offers considerable promise for improved therapy in septic shock if it could be determined with reasonable certainty exactly what should be inhibited or antagonized. It seems likely that this will not be achieved until we alter our research endeavors to focus on the ultimate model of human infections, that of infections in man, or on other models of infection, rather than focusing on results observed after endotoxin challenge.

DISCUSSION

This limited review is intended solely as an overview of a clinician's perspective of the applicability of the extensive investigative efforts on septic shock to the care of patients with Gram-negative bacteremia and shock. It is not intended as criticism of various investigations or investigators, but is specifically intended to emphasize the vast number of "in vitro" studies and our continuing lack of definitive information concerning the basic mechanisms responsible for the development and course of shock. As one who has watched the "ebb and flow" of popularity concerning the importance of a variety of postulated effectors and mediators, it is disappointing to this clinician that, to date, none have resulted in new therapies clearly shown to significantly improve survival rates in patients with septic shock. As stated earlier, it seems clear that newer antimicrobial agents will probably not further increase the salvage rates in those with septic shock and that further improvement will depend on improved methods of management of crucial hemodynamic derangements. The primary purpose of this review is to urge greater attention to the intact whole patient and less focus on "in vitro" phenomenology, unless this is clearly established to have clinical relevance.

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DISCUSSION PERIOD WITH DR. McCABE

DR. BRAUDE: Dr. McCabe and I have been arguing about the importance of endotoxin and shock associated with Gram-negative bacteremia for about 25-years. I do want to respond to a couple of the points that he made in his lecture to indicate that there is still a good reason to believe, I think, that endotoxin plays an important role.

Dr. Gerhardt's experiments on the surface would seem to be very appealing as evidence against the importance of circulating endotoxin in Gram-negative bacteremic shock. I have always interpreted these differently. I thought that they confirmed our studies, which showed that circulating endotoxin was degraded in size to the point where it could pass through membrane filters, molecular weight range was consistent with postulated sizes retaining activity.

We also did the same thing with dodecylsulfate. Dodecylsulfate, can reduce the particle size of LPS without affecting it's toxicity. That particle size would pass through membrane filters.

The most striking clinical evidence, for the importance of endotoxin, are conditions of bacteremia. Bacteria with endotoxin on their surface are circulating through the body fluids.

I don't think it is necessary to postulate that endotoxin is shed from the bacteria, as you suggested. When you inject dead Gram-negative bacteria into the circulation, or when you kill bacteria with antibiotics, these organisms are circulating and continue to be extremely toxic.

One of the best examples of this is contaminated blood transfusion reactions with cold-growing bacteria that can't multiply in the blood. Endotoxin on organisms can throw people into the worst kind of transfusion reactions, fatal reactions with classical shock identical to that which we see in Gram-negative septicemias.

I think that the striking association between the occurrence of shock and Gram-negative organisms in the blood is strong circumstantial evidence in favor of endotoxin on the organism as being important.

With regard to the BCG studies, I thought that one point that was overlooked in the BCG studies is that BCG increases sensitization to free LPS. No studies have been done to show that BCG increases susceptibility to killed Gram-negative bacteria containing endotoxins on their surface. I don't think it does based on observations made in our laboratory. Clearance of circulating free endotoxin is affected in BCG mice, but I don't think that clearance of bacteria is.

So I think that there are a number of reasons to believe that under some circumstances we can make a case against endotoxin, as Dr. McCabe has done. But under other circumstances, particularly when you are dealing only with the concept of endotoxin on the surface of the organism that is circulating, it is pretty hard to exclude it as a factor in the development of shock.

DR. McCABE: I would certainly agree with your first point, that we have discussed this for some 20 odd years.

Pegarding your point about the Quarrels and Gerhardt study, we have tried to dissociate LPS and maintain it in a molecular size aggregate of less than 100,000. At that size, however, we haven't been able to show toxicity.

I think your point about whole bacteria is really the point I am trying to make. I don't doubt that endotoxin contained in whole bacteria may play a role.

The reason I make this point is that there has been a tremendous tendency to equate Gram-negative bacteremia with diphtheria as a toxin produced disease. Yet, the one thing we clearly can show is we can reduce lethality by killing the bacteria with antibiotics.

So please don't misunderstand me. I think that intact bacteria with LPS on it, indeed, is true. Whole bacteria are much more effective in activation of complement than the equivalent amount of LPS released from them. Bacteroides fragilis has an LPS that isn't biologically active and, at least, in our Gram-negative bacteremias it is associated with the frequency of shock comparable to that of E. coli.

We really both make the same point, that the bacteria are critical to the pathophysiology of the individual and that it is important to recognize that, rather than individual components. Also, we get into a bit of trouble when we extrapolate from animal models to man because there is so much difference.

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COLONIZATION ABNORMALITIES AS A MAJOR CAUSE OF TRAUMA-INDUCED INFECTIONS

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INTRODUCTION

In spite of many improvements in the treatment of trauma patients, infection continues to be the major obstacle to improved results in the care of these individuals. In fact, among stabilized severely injured patients, infection is second only to head injury as a cause of death.[1] Uncontrolled infection is the most crucial causative factor in the evolution of organ failure among compromised patients.[2]

The mechanisms that provide the opportunity for otherwise nonpathogenic microorganisms to establish debilitating and life-threatening infections after severe single or combined injuries have been the subject of extensive studies. Many of the latter have concentrated on the important areas of microbial toxins and suppressive effects of trauma on the natural humoral and cellular immune defenses of the host.

In recent years, another facet of host-microbial interactions has received increasing attention. This facet concerns the phenomenon of abnormal colonization of mucosal surfaces by microorganisms. Abnormal colonization and defense suppression are two key events for the establishment of opportunistic infections following direct and mediated effects of severe injury (Fig. 1).

This report will describe mechanisms influencing the colonization of mucosal surfaces by microorganisms, show how this process may be altered by trauma, and propose some means by which colonization may be manipulated for the benefit of the patient.

NORMAL COLONIZATION

Normal mucosal surfaces are relatively resistant to colonization by pathogenic bacteria. For example, when volunteers gargled a challenge consisting of over 10^8 cells of Escherichia coli, Klebsiella pneumoniae, or Proteus mirabilis, these inocula were reduced to less than 1% of the original number by 3 h post exposure.[3] Such resistance is conferred by fluids and secretions bathing the mucosa, which contain substances that damage or coat the microorganisms so that they cannot make contact with receptor sites on cell membranes.[4] These chemical defenses are augmented by the physical removal mechanisms, which include peristalsis, mucociliary action, etc. Colonization resistance also depends on the contribution of indigenous flora, which occupy binding sites and produce metabolites that regulate colonization by pathogens.

When these normal mucosal defenses are impaired or are overwhelmed by large numbers of microorganisms, then opportunistic pathogens may be able to bind to epithelial cells and initiate disease processes. Even with reduced mucosal defenses, successful pathogens must be able to locate the epithelial cell through motility and chemotaxis, have appropriate adhesins to interact with mucosal receptors, and be able to multiply rapidly so that they will not be removed as epithelial cells are shed.

Hydrophobic interactions between molecules on the surface of the bacterial cell and phospholipid molecules of the host cell membrane overcome the repulsive negative charges on bacterial and host cell surfaces. Permanent binding, however, is effected by specific interactions between adhesin molecules of bacteria and their complementary receptor molecules on host cell membranes. Bacterial adhesins are often associated with hair-like surface appendages called fimbriae on Gram-negative bacteria or fibrillae on Gram-positive flora. Bacterial colonization can be further stabilized by secretion of glycocalyx material, which cements the colony to surfaces and impairs phagocytic removal.[5]

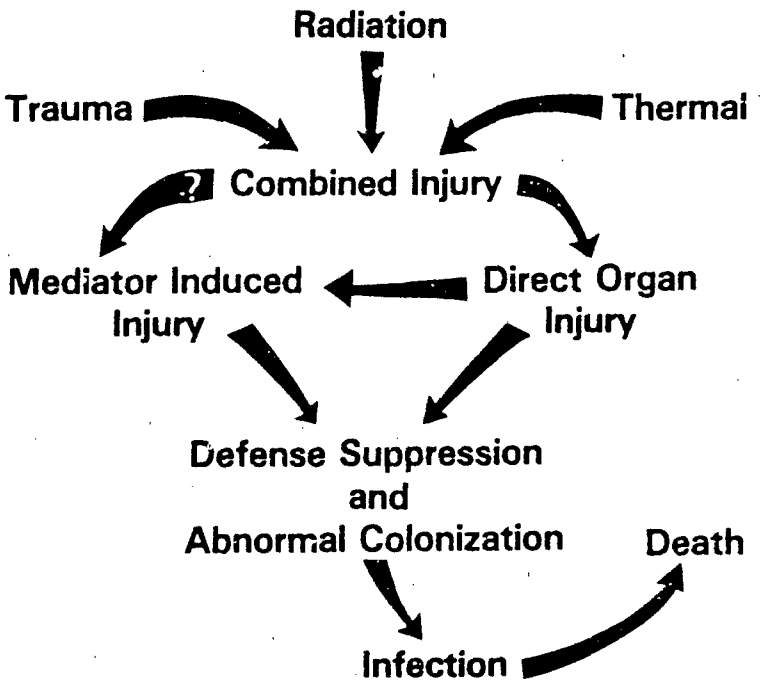


Fig. 1. Infection following injury as a consequence of defense suppression and abnormal colonization

ABNORMAL COLONIZATION OF THE GASTROINTESTINAL TRACT

The gastrointestinal tract, which includes mucosal surfaces of the oropharyngeal cavity and the intestines, is the major source of bacterial infections in the compromised host.[6-9] Bacteremia is usually associated with colonization of the gastrointestinal mucosa by organisms such as Pseudomonas aeruginosa and K. pneumoniae, which are frequently acquired subsequent to patient hospitalization.

OROPHARYNGEAL COLONIZATION. Impairment of the normal pharyngeal clearance mechanisms could be an important initial step in the development of pneumonia due to Gram-negative bacilli. The prevalence of these bacteria among the normal oropharyngeal flora has been found to rise markedly in hospitalized patients.[10] This event did not correlate with antibiotic administration, inhalation therapy, or duration of hospitalization, but it did correlate with severity of illness.

More recent studies provide some insight into how severe illnesses may affect the colonization resistance of a host. Woods et al.[11] noted that a correlation between the *in vitro* adherence of *P. aeruginosa* on upper respiratory tract epithelium and the colonization of the tract by this organism. For example, the respiratory tracts of cystic fibrosis patients, in contrast to normal individuals, are colonized with *Pseudomonas*. Buccal epithelial cells from these patients bind 19.1 bacteria per cell versus 2.3 bacteria per cell from normal individuals. Interestingly, the increased binding capacity of buccal epithelial cells from cystic fibrosis patients varied directly with the loss of the protease-sensitive glycoprotein, fibronectin, from the cell surface. This loss of fibronectin is thought to be associated with increased levels of salivary proteases.

Woods' findings are also pertinent to other compromised host models. In a study of 12 patients in an intensive care unit, Woods found that their buccal epithelial cells bound more *P. aeruginosa* than did their uncolonized controls.[12] Similarly, in patients with coronary bypass surgery, salivary protease activity and bacillary adherence increased as cell surface fibronectin decreased.[13] These changes in adherence capacity of cells from bypass patients were transient in uncomplicated cases, and had largely returned to normal by the third post-operative day.

Some of Woods' findings may be explained by reduction in salivary flow, which can occur in hospitalized patients. It has been associated with increased proteolytic activity and reduced intraoral pH, both of which can increase the susceptibility of epithelial cells to binding by Gram-negative

bacteria.[14] Lack of adequate salivary flow could also affect the colonization due to loss of factors such as immunoglobulin A and direct antibacterial components such as lysozyme contained in this fluid.

Other injury to epithelial cells can also be involved in opportunistic adherence. Desquamating cells of murine tracheas injured by influenza virus infection or by endotracheal intubation were found to bind Pseudomonas more readily than did cells from normal animals.[15] Colonization of the respiratory tract by Pseudomonas is also enhanced in rats with microulcerative lesions caused by hexamethylphosphoramide.[16] The significance of changes in the physiologic status of mucosal cells can be influenced by unique binding characteristics of various organisms. For example, Pseudomonas binds readily to mouse tracheas injured by 0.1 N HCl for 15 min, but K. pneumoniae and E. coli bind more effectively to normal tracheal cells.[17] The unique binding characteristics of Pseudomonas may contribute significantly to the importance of this organism in compromised patients.

INTESTINAL COLONIZATION. Changes in the colonization affinity of the mucosal surface of the lower gastrointestinal tract have not been studied as well as those of the oropharyngeal region. From quantitative studies of intestinal colonization, however, it is known that microbial populations in this region are very sensitive to changes in host physiology. Injury, environmental stress, and dietary stress in animals and even emotional stress in humans cause significant alterations in numbers of various fecal flora.[18-21]

We have examined changes in populations of intestinal flora in irradiated rats that could predispose the animals to sepsis, by comparing the numbers of facultatively anaerobic flora in ileal homogenates at various times after lethal (1000 rads) and sublethal (500 rads) ^{60}Co radiation.[22-23] The numbers of bacteria declined from normal levels in animals given either 500 or 1000 rads of radiation (from 239.0 to 11.7 and 39.4 x 10^4 bacteria per gram of intestine, respectively). At the lower radiation dose, the numbers of these bacteria began to return to normal levels after day 7 post radiation, and were approximately normal by 11 days after radiation. In contrast, by day 11 in rats given 1000 rads ^{60}Co, these organisms had colonized the ileum in numbers far above normal (740.7 x 10^4 bacteria per gram of intestine).

Evidence for the importance of colonization resistance and the presence of injury-induced alterations in the intestinal mucosal environment have been obtained from further studies of ilea from lethally and sublethally irradiated rats.[22-23] Scanning electron microscopy (SEM) was used to study a group of indigenous flora known as the segmented filamentous microflora (SFM). These presently uncultivatable organisms are intimately associated with the ileal epithelium of normal rodents. These organisms were recently associated by others with colonization resistance by quantitating them *in situ* with transect line analysis of SEM images.[24] Colonization by the SFM correlated significantly with development of host resistance to fatal infection by oral challenge with *Salmonella enteritis*.

The SFM were associated earlier with colonization resistance in irradiated rats.[22,23] One day after sublethal or lethal radiation, SFM were absent from the intestinal villi (Fig. 2). By 4 days after radiation, however, villi in rats receiving sublethal radiation were colonized by normal numbers of the SFM. In rats given lethal radiation, the SFM were still absent at 11 days post radiation. Thus, lethal injury causes a response that prevents some normal flora from colonizing the ileum. The loss of these flora may contribute to subsequent overgrowth by opportunistic pathogens.

The mechanisms responsible for ecological changes in the ileum post radiation are relatively unknown. One important mechanism could involve quantitative and/or qualitative changes in the mucous gel that bathes intestinal epithelium. This material is secreted by specialized epithelium called goblet cells, and consists of 5-20% high molecular weight glycoproteins. Recent new fixation techniques make it possible to visualize this layer with SEM.[25] When this is done, the mucous gel can be easily determined to be the major ecological niche in the intestine. Most microorganisms are found here. Study of the fate of this substance after trauma may help explain changes in bacterial populations.

There are some reasons to think that the mucous gel could be altered after trauma. Mucus production in the gastrointestinal tract is increased during burn shock.[26] Furthermore, in study of changes in intestinal permeability following radiation injury[27], full-thickness dermal wounds[23], or endotoxin

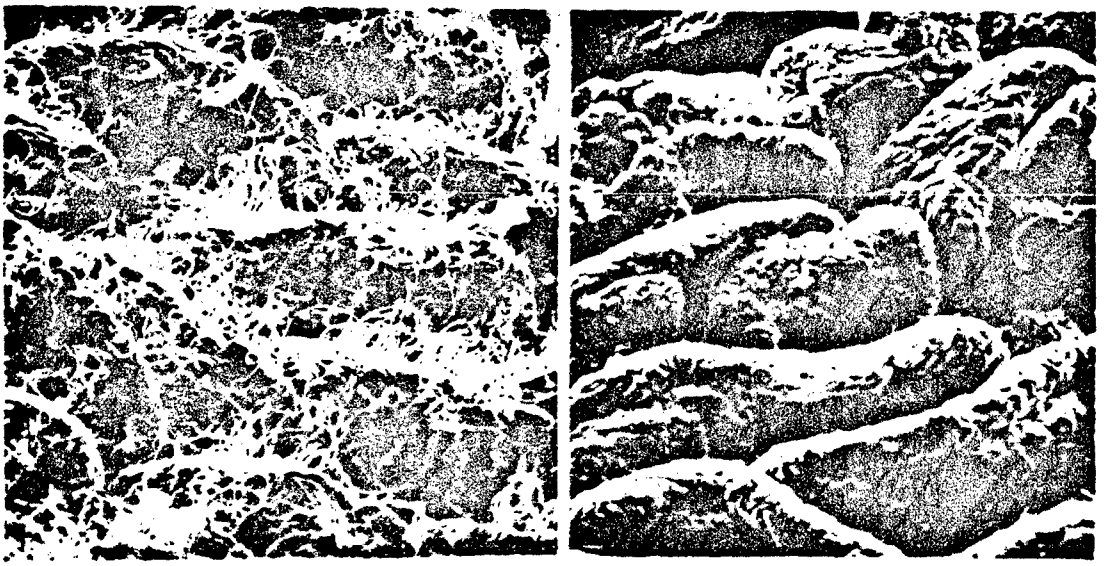


Fig. 2. Rat ileum before (left) 1 one day after exposure of the animal to 1000 rads ^{60}Co radiation.

shock,[28] it was found that the tight junctional barriers (zonula occludens) associated with goblet cells are often disrupted. These intercellular barriers between adjacent absorptive epithelial cells are always intact. These data indicate that a variety of injuries have physiologic effects on goblet cells. Since some of these injuries would not be expected to directly affect intestinal cells, their effects on goblet cell tight junctions must be mediated by factors secreted elsewhere. The possibility that soluble mediators may influence goblet cells is not surprising, since secretion by these cells is under parasympathetic control,[29] and vasoactive amines such as histamine can disrupt goblet-cell-associated tight junctions.[30]

More direct study of mucous gel affinity for bacteria in compromised subjects is now possible. Dr. Paul Cohen at the University of Rhode Island has developed an *in vitro* mucous gel adherence assay,[31] which we have adapted and begun to use to determine changes in binding affinity in trauma models. The gel material for this assay was obtained by removing small intestines from 10 C3HeB/FeJ mice at 0,1,3,5,7,9, and 11 days after 1000 rads ^{60}Co. The intestine

was rinsed with phosphate-buffered saline (PBS, pH 7.3), slit lengthwise, and scraped in cold PBS. All materials collected and pooled from the 10 mice were centrifuged twice for 20 min at high speed to remove all cell debris. The protein concentration of the final supernatant was adjusted to 3.5 mg/ml. A quarter ml, or approximately 0.9 mg, of protein of this material was put into each well of a 24-well Linbro plastic tissue culture plate overnight at 4°C. The next day the wells were washed three times with PBS, and this crude preparation was then used as the substrate for the binding of tritium-labelled bacteria.

Radiolabelled bacteria were washed twice with PBS, adjusted to a standard cell concentration of approximately 10^8 cells/ml, and 1/4 ml (or about 1×10^8 cells total) was allowed to incubate on the mucous gel for an hour at 37°. The non-adherent bacteria were removed and the well washed three times with PBS. Adherent bacteria were then recovered from the wells by adding 0.5 ml of sodium dodecyl sulfate to each well and reincubating the plates for 3 h at 37°. The radioactivity remaining in each well after washing with PBS was used to calculate the number of cells that adhered to the mucous gel.

With this new adherence assay, we have detected changes in binding affinity of gel preparations seen after lethal radiation (Fig. 3). By day 7 post-radiation, the number of receptors in the mucin for the nonpiliated laboratory E. coli strain K12 had risen steadily to six times the normal level. This suggests that lethal radiation can alter intestinal mucoproteins so that organisms that normally cannot attach to the mucous gel or that do so poorly in competition with indigenous flora are able to attach and colonize more effectively. When the E. coli K12 contained the plasmid for the K88 pilus, normal binding was higher than in the parent strain. There was an initial drop in binding capability on day 1 after radiation, but over the next 10 days the number of cells bound by the mucous gel remained at normal levels.

Post radiation binding by two strains of P. aeruginosa to mucous gel has also been studied. The binding affinity of mucous gel for the PAKS-10 strain of Pseudomonas increased dramatically after radiation, and remained elevated through day 11. In contrast, adherence of the E64 strain of Pseudomonas was increased only at day 1 after exposure. Abnormal colonization of the

underlying epithelial layer by opportunistic pathogens may require only 1 or 2 days of optimal attachment to the mucous gel. Changes in the amount of mucous gel secreted could even magnify differences observed in binding affinities. Studies are presently under way to quantitate the mucous gel layer following lethal radiation.

COLONIZATION OF SITES OUTSIDE THE GASTROINTESTINAL TRACT

Effects on colonization such as seen in the gastrointestinal tract could also occur on other mucosal surfaces and contribute to post trauma infections. This may be particularly true of the urinary tract, where catheters provide a portal for the introduction of pathogens. Although the effects of trauma on the urinary tract epithelium have not been studied, considerable evidence is available to associate urinary tract adherence with infection. Schaeffer et al.[32] found that the susceptibility of women to urinary tract infection is associated with widespread fluctuating changes in the adhesive characteristics of their epithelial cells taken from other sites. The adherence of bacteria to vaginal or buccal cells was greater in patients with recurrent urinary tract infections than in normal individuals.

Changes in binding affinity could be due to changes in cell membranes themselves or in their protective coatings. For example, transitional cells lining the urinary bladder secrete glycosaminoglycan (GAG), which reduces E. coli adherence.[33] When GAG is removed from the urinary bladder epithelium, adherence increases, but returns to normal as it is resynthesized. Hormones may alter cell membrane receptors for bacteria. For example, incubation of HeLa cells with estrogens enhanced the adhesion of E. coli and S. aureus.[34]

PREVENTION OF ABNORMAL COLONIZATION

Our present understanding of host and microbial factors affecting the colonization of mucosal surfaces can be used to propose a number of approaches to infection control.

COMPETITIVE INHIBITION. Purified adhesin or receptor materials or their analogues can be used as competitive inhibitors of bacterial adherence. For example, Aronson et al. used intravesicular instillation of mannose to control

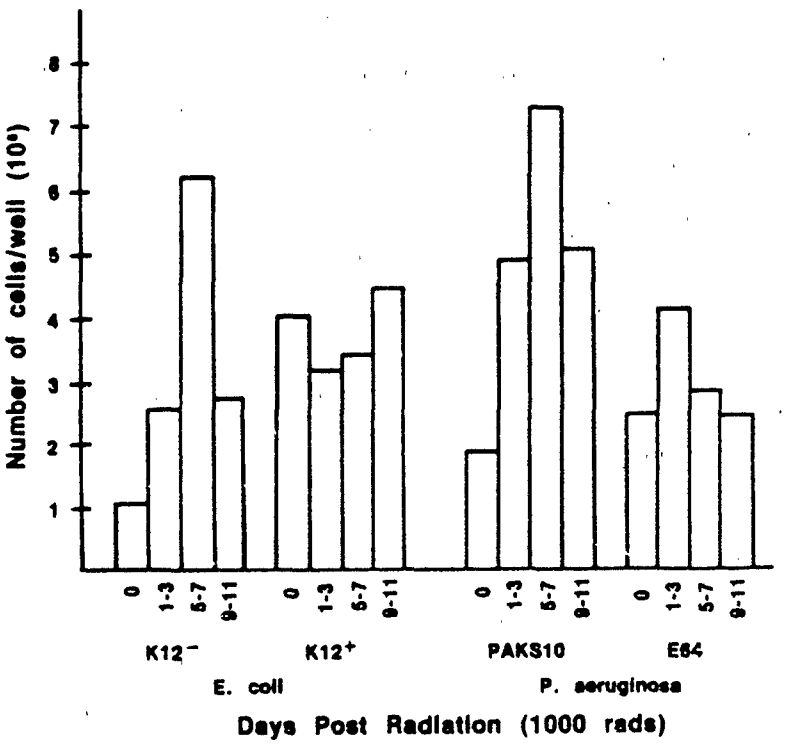


Fig. 3 Number of bacteria adherent to mouse mucus specimens obtained at intervals after exposure of the animals to 1000 rads ^{60}Co radiation

urinary tract colonization of mice by mannose-sensitive E. coli.[35] Although 70% of mice given phosphate-buffered saline were colonized by E. coli, only 20% of the animals given methylmannoside were colonized.

ANTIBIOTIC INHIBITION. Administration of sublethal concentrations of antibiotics can suppress the formation and expression of bacterial adhesins. Adhesion of Streptococcus pyogenes is blocked by low concentrations of penicillin through loss of the adhesin necessary for the bacteria to bind to human buccal epithelial cells.[36] Sublethal doses of either penicillin or streptomycin can prevent the formation and expression of adhesins of E. coli.[37]

Even when fimbriae are present on these bacteria after antibiotic treatment, they may be ineffective due to amino acid substitutions induced by the effects of antibiotics on reading of messenger RNA.

VACCINES. Vaccines against specific surface components involved in adhesion to mucosal surfaces would be useful in prevention of infection. This approach has shown promise for preventing gonococcal infections in normal humans.[38] The antigenic diversity of binding components of organisms would make this approach difficult for controlling many opportunistic infections. It is possible, however, that receptor-binding domains among the fimbriae of various organisms will prove to have common antigens, and then vaccines prepared from peptide fragments containing the common bacterial cell membrane-binding region could be broadly protective.

SELECTIVE DECONTAMINATION. The process of using antimicrobial agents that inhibit colonization by potential pathogens but leave benign flora intact will be discussed in detail by Dr. van der Waaij. This method has already been used successfully in patients to maintain a flora that reduces opportunities for colonization by other organisms.

STABILIZATION OF CLEARANCE MECHANISMS. Restoration or preservation of colonization-regulating fluids on mucosal surfaces may become an effective means of reducing colonization by opportunistic pathogens. Since fibronectin, which blocks many binding sites found on cell surfaces, can be lost as a consequence of proteolytic activity,[11,13] it may be possible to inhibit protease activity in secretions and/or artificially restore cell surface fibronectin. Simple means such as increased mastication to enhance salivation in hospitalized patients may enhance clearance mechanisms in the oral-pharyngeal region. If mucus secretions in the intestine are significantly altered by various injuries, it may someday be possible to manipulate them pharmacologically.

CONCLUSION

The importance of microbial adhesion to mucosal surfaces has been recognized only in recent years. The role of this event in colonization, a

major predisposing factor to subsequent infection in compromised hosts, seems well established. It is now imperative that host and microbial factors responsible for colonization by opportunistic pathogens become better understood through careful study. As we learn more about the phenomenon of colonization after trauma, we can begin to exploit them to prevent infections from being initiated in a host already compromised in its ability to deal with them.

ACKNOWLEDGEMENT

The authors appreciate the helpful editing of this manuscript by Dr. H. Morgan and Ms. D. Boyle for editorial assistance.

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DISCUSSION PERIOD WITH DR. WALKER

DR. ALTURA: About 20 years ago we published that tetracycline, given to normal rats, 3 days before trauma increased mortality.

Secondly, we found a marked depression of the reticuloendothelial system. These results have been confirmed in PMN's from patients and a number of other studies in Germany and Sweden over the last 20 years.

Three years ago we reported that antibiotics Dr. van der Waaij used (ampicillin, kanamycin and tetracycline) increased mortality in normal control rats if given 3 days before superior mesenteric arterial occlusion bowel ischemic shock and Noble-Cobb Drum trauma. Interestingly enough, the reticuloendothelial system was depressed in normal animals after 3 days of pretreatment with dosages similar to what would be given to human beings. So I wonder whether or not the results you have shown may not be a reflection of what is happening to phagocytic events.

One further thing: Using specific pathogen-free rats, where we decontaminated about 85 percent of the normal flora, we also found a depression of the phagocytic system. They were highly susceptible to hemorrhage, trauma,

and bowel ischemia. We reported these findings about 10 years ago. In all animals studied, there was an inability to show compensatory rebound after bleeding or trauma of blood pressure.

DR. WALKER: One point that comes out of what you said is that bacteria are basically good. They don't necessarily have to be villains. It is only when something goes wrong that we can't live with them any more, and then we are in trouble. That is why it helps to be able to get endogenous bacteria back in line so some problems can be prevented. The other point you mentioned is that we have to consider using antimicrobial approaches that are not just affecting the microorganisms but are also affecting the physiology of the host.

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INFECTION PROPHYLAXIS IN LARGE PATIENT POPULATIONS WITH COMBINED INJURY

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INTRODUCTION

On August 9, 1945, 201 prisoners of war from the Royal Army of the Dutch East Indies were imprisoned in camp FUOKA-14 in Nagasaki. This camp was located in a suburb called Saiwaimachi, about 1650 meters from the hypocenter of the explosion of "Fat Man", the plutonium bomb that exploded that day (Fig. 1). The POW's had been put to work in the machine shop and casting shop at Mitsubishi Shipyard. These men were in bad health at the moment of the explosion, due to their heavy labor and malnourished condition. Four POW's were killed immediately by the explosion, and 31 were seriously wounded. The low mortality may have been due largely to the rapid action of the surviving POW's who pulled their colleagues from ruins of buildings and from underneath wreckage and rubble before it caught fire. The 31seriously wounded men were carried into the hills in the south on improvised stretchers.

It was largely due to this rapid rescue activity by POW's and the presence of a small hidden reservoir of medicines taken a few months previously from American Red Cross food packages, that only five of them died as a result of (combined) trauma due to the explosion, i.e., due to blast, burn, and radiation. The POW's stayed in the area of Nagasaki until September 12, so they may have received some additional radiation. Little medicine and simple hygienic measures, such as the disinfection of bandages in boiling water before reuse and the preparation of food of better quality than during the preceding months, may be of importance in explaining the relatively low mortality of only 4%. The mortality among the Japanese population in the same area during the explosion was about 28%.[1]

This short summary was collected by Dr. Stellingwerff[2] from several diaries of POW's who survived the Nagasaki A-bomb and from personal interviews of a number of them. It may support my view that some form of simple medical help may be of great importance for A-bomb victims. If people survive the direct effect of a considerably heavier A-bomb than used in Nagasaki, their survival may be increased by simple measures such as (a) direct rescue of wounded by "healthy" survivors in the area to limit death due to fire and (b) provision of simple first aid, which helped the POW's in Nagasaki to survive. Survival from the direct effects of an A-bomb explosion will be possible only at a certain distance from the hypocenter. This distance will be proportional to the power of the explosion.

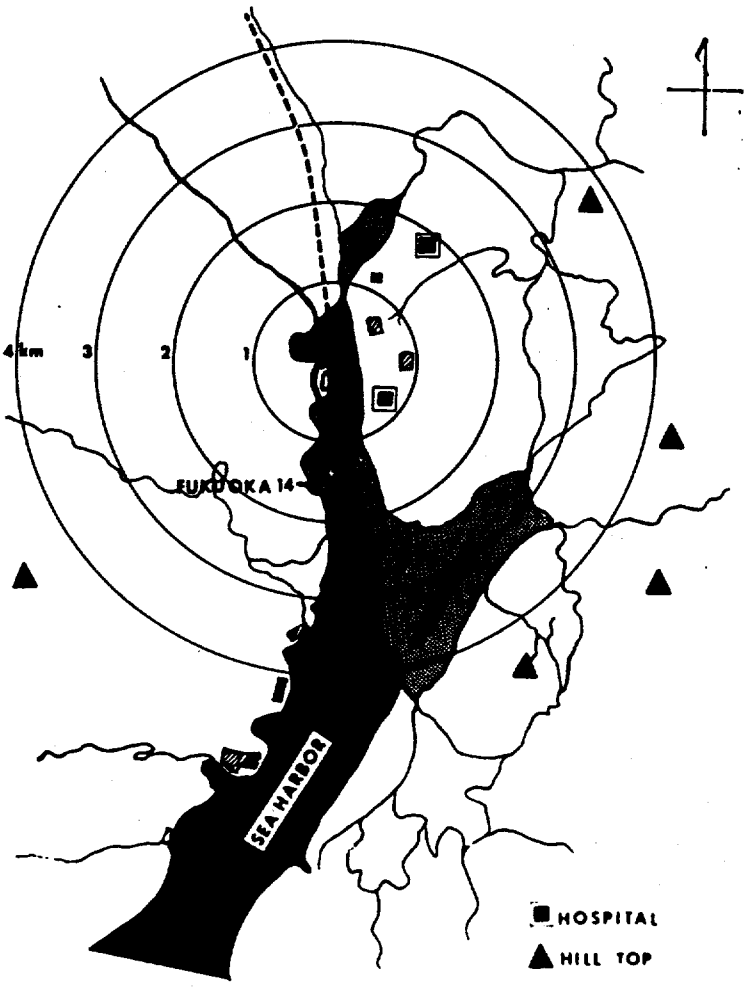


Fig. 1. Zones beyond the hypocenter of the explosion of "Fat Man" at Nagasaki

RADIATION DOSE DISTRIBUTION IN NAGASAKI DUE TO A-BOMB EXPLOSION

Last year at the VIII Symposium on Infectious and Parasitic Diseases in Stockholm, I presented a survey of what presently could be done to limit infections among A-bomb victims or other categories of mass casualties.[3] This survey was also based on information concerning the physical, medical and social effects of the atomic bombings of Hiroshima and Nagasaki.[1] It was shown that blast and flash burn victims were seen over a much greater area (at greater distance from the hypocenter) than low radiation doses of 100 rads or less (Table 1). The casualty incidence in Nagasaki was 50% in a zone in which radiation was much less than 100 rads.

TABLE 1. DATA[a] PRESENTING RETROSPECTIVE OBSERVATIONS IN NAGASAKI RELEVANT TO THE DISCUSSION OF FIRST AID A-BOMB VICTIMS

DISTANCE FROM HYPO-CENTER IN M'S	"TENTATIVE 1965 RADIATION DOSE" IN RADS		INCIDENCE (%) OF INJURIES		INCIDENCE (%) OF ESTIMATED RADIATION ACQUIRED		
	X-RAYS	NEUTRONS	BLAST	BURN	CASUALTY	MORTALITY	
1000	888	35.9	75	25	90	70	"lethal"
1100	588	19.5	--	--	--	--	
1200	391	10.6	60	27	85	65	
1300	262	5.8	--	--	--	--	
1400	176	3.2	--	--	--	--	
1500	119	1.7	55	29	80	40	
1600	80.7	1.0	--	--	--15[b]	--4[b]	
1800	37.6	0.3	--	--	--	--	
2000	17.8	0.1	35	33	57	12	
3000	0.5	0	22	30	17	0	No
4000	0	0	19	8	7	0	Significant Radiation

[a] derived from (1)
[b] camp FUOKA 14

Regardless of the explosive power of an A-bomb, the zone in which people survive the direct effects of the explosion but are sublethally irradiated or wounded or both, is unlikely to be much wider than it was in Hiroshima and Nagasaki, i.e., an outer zone of about 5 km deep. Long-term survival will be possible only in those in whom the total-body radiation dose (gamma rays or neutrons) does not exceed the dose that causes severe damage to the bone marrow but still leaves a sufficient number of stem cells for rapid repopulation. Without advanced medical care, acute radiation doses of 700 rads and more are inevitably lethal due to infection and/or hemorrhage. Partial irradiation (when part of the blood forming bone marrow is shielded during an A-bomb explosion) may provide a chance of survival for people in zones in which radiation is stronger. They may possibly survive a dose as high as 800 rads, particularly when the shielding covers the abdomen, with the intestines and the iliac bones containing bone marrow. The dose range of 600 rads down to less than 10 rads, which will likely be survived by the majority, will in all cases exist only over a very narrow zone of a few hundred meters. The distance of this zone from the hypocenter will depend on the size of the bomb. Heavy explosions of, for example, tenfold the exposion that has occurred above Nagasaki, will have this zone at a proportionally greater distance from the hypocenter. Consequently, following a megaton bomb explosion, the total surface of the zone for potentially long-term surviving radiation victims will be much larger than in Nagasaki, although the zone will not be wider. This involves only fully exposed persons. As mentioned above, individuals who are partially or completely shielded during the explosion (being, for example, inside a solid concrete building) may have a chance to survive when they are closer to the hypocenter.

As in Nagasaki, long-term survivors of an A-bomb explosion are to be expected only in the zone in which they may receive 600 rads or less. Mutual assistance as well as rescue and aid by residents of cities and villages at greater distance from the hypocenter (upwind from the bombed area) may be of great importance in lowering the mortality. If this is to be realized, it would be wise to inform the public about the real risk for health and life after exposure to low radiation doses. If an atomic weapon is ever used by any nation, the fear and panic in the target area may be much greater than those

that occurred in Hiroshima and Nagasaki. As a consequence, mortality among the wounded, the combined-injury, or (low-dose) radiation victims who survive the direct effect of the explosion, may considerably exceed the mortality reported in both cities of Japan. We should aim for the opposite.

SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT: A METHOD TO PREVENT BACTERIAL INFECTIONS

The anaerobic fraction of the digestive tract microflora of animals and man has been found to be of key importance for the control of the colonization pattern of the digestive tract by potentially pathogenic microorganisms such as Escherichia coli, Klebsiella species, Pseudomonas species, and Gram-positive cocci.[4,5] Enteric pathogens like Salmonella, Shigella, and Vibrio cholerae also are negatively influenced by the autochthonous anaerobic intestinal flora.[6-9] Anaerobic bacteria form a strong barrier to colonization by pathogenic and potentially pathogenic bacteria. This phenomenon is called colonization resistance (CR).[4] The CR is susceptible to a number of antibiotics.[10]

Selective decontamination (SD) of the digestive tract aims at (a) the selective elimination of the potentially pathogenic and the pathogenic bacteria from the digestive tract and (b) the prevention of colonization by these species. Such selective growth suppression can be obtained by oral administration of a combination of antimicrobial drugs that do not affect the CR (anaerobic) flora. SD is achieved only when such treatment causes the disappearance of potential pathogens and pathogens from surveillance cultures of feces and oral washings. If this is not achieved, only selective suppression of opportunistic and pathogenic flora is obtained.

Selective decontamination appears to be of practical importance in preventing infection in patients with acute leukemia or other groups of patients who suffer from severe granulocytopenia. SD treatment can be continued for weeks and, if necessary, for months. Since the CR flora are maintained, SD does not require isolation precautions other than treatment under hygienic hospital circumstances. As long as the anaerobic flora are not affected by

antibiotics used for SD, the threshold for a "take" by pathogens is high; i.e., high numbers of bacteria (of most species, 10^6) must be ingested to establish colonization. Information about which antibiotics do lower the CR flora (the anaerobes) and which do not, data about daily doses required for successful SD, and other technical details, fall out of the scope of this review. Detailed information, however, is reported elsewhere.[11-18] SD has been found to be an impressive means for infection prophylaxis in humans and animals when applied shortly after sublethal total-body irradiation.[19]

AN ANIMAL MODEL FOR THE STUDY OF INCIDENCE AND SPREAD OF INFECTION

Animal experiments have been conducted to investigate what may occur among civilians who survive the direct effects of an A-bomb explosion but have received radiation and who are treated with an antibiotic(s) that does or does not affect the CR flora. Two groups of randomly bred Swiss female mice, 12 weeks of age, were X-irradiated with 700 rads and placed 15 to a cage. The animals in each cage shared one drinking bottle and a hopper with pelleted food.

Two cages each with 15 animals were not irradiated, but were given ampicillin (CR-decreasing) in the drinking water (0.5 mg/ml),[20] and treated with either nalidixic acid (CR-indifferent) in the drinking water (1 mg/ml[21] or polymyxin B in the drinking water (1 mg/ml).[22] In four of these eight cages (one of each treatment group), one ear-marked animal was orally contaminated the day before treatment and irradiation with 10^9 ampicillin and nalidixic acid-resistant Escherichia coli. In the remaining four cages, one ear-marked mouse was orally contaminated the day before treatment and radiation with 10^9 Pseudomonas aeruginosa. In all cages individual sampling of feces was attempted every other day. In mice that died, heart blood was taken aseptically for culturing.

The results of the study can be divided into epidemiologic results (Fig. 2 and 3) and results concerning survival (Figs. 4 and 5).

The spread of both E. coli and Ps. aeruginosa was rapid in the ampicillin-treated group. This obviously correlated with the decreased CP in these animals. Spread of both of the Gram-negative bacilli among cage mates in the

control group as well as among the nalidixic acid-treated mice was seen only in the Ps. aeruginosa-contaminated groups (Fig. 3). The fact that the contaminant strains as well as the endogenous Gram-negative bacilli were sensitive to polymyxin B may explain why these bacteria were not cultured from the feces of polymyxin-treated mice.

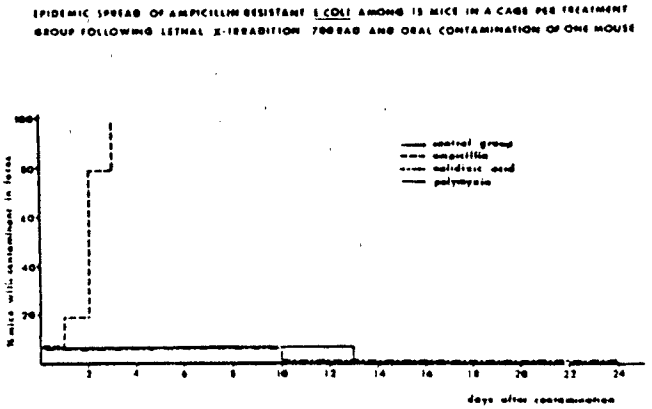


Fig. 2. Epidemic spread of ampicillin resistant E. coli among 15 mice in a cage per treatment group following lethal X-irradiation (700 rad) and oral contamination of one mouse

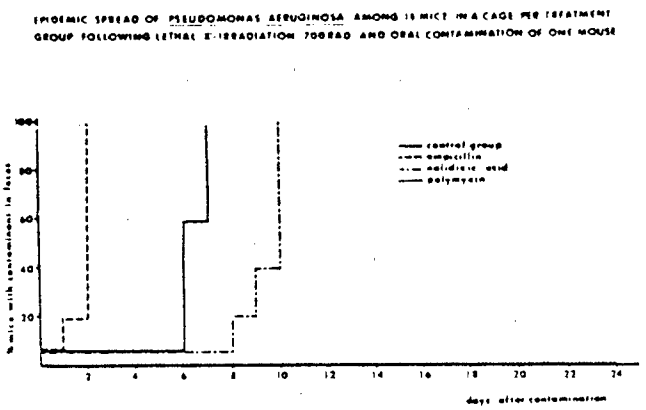


Fig. 3. Epidemic spread of Pseudomonas aeruginosa among 15 mice in a cage per treatment group following lethal X-irradiation (700 rad) and oral contamination of one mouse

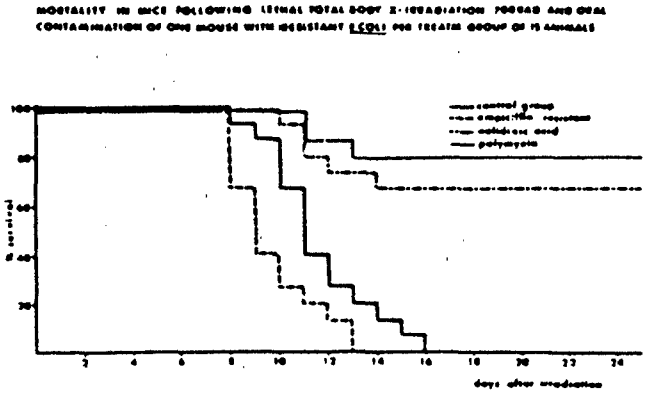


Fig. 4: Mortality in mice following lethal total body X-irradiation (700 rad) and oral contamination of one mouse with (resistant) E. coli per treatment group of 15 animals

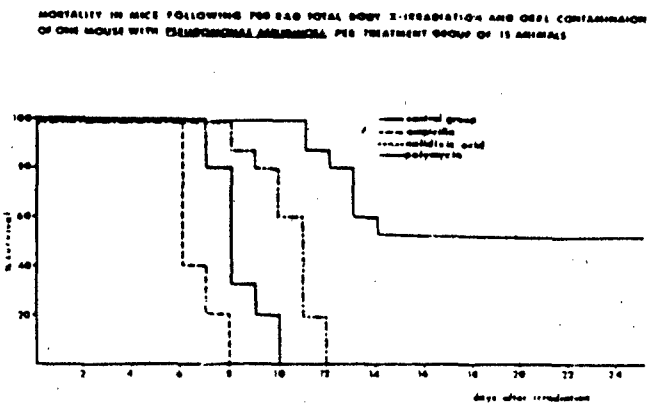


Fig. 5. Mortality in mice following 700 rad total body X-irradiation and oral contamination of one mouse with Pseudomonas aeruginosa per treatment group 15 animals

The influences of overgrowth in the gastrointestinal tract of ampicillin-treated mice by the resistant potentially pathogenic bacteria (E. coli and Ps. aeruginosa) on mortality following X-irradiation (Figs. 4 and 5) is well known.[23,24] It is, however, perhaps not realized that the post-irradiation therapy with CR-decreasing antibiotic treatment may enhance mortality as it did in the animal model described here. When irradiated individuals become heavily colonized (overgrown) by resistant potentially pathogenic organisms, the almost inevitable infection will soon become lethal if specific antibiotic therapy is not rapidly started. The effect of CR-decreasing treatment is more dramatic if a more pathogenic species such as Ps. aeruginosa is involved than if the digestive tract of irradiated individuals becomes loaded with massive numbers of E. coli.

Selective decontamination with nalidixic acid or polymyxin on the other hand, reduced mortality in our mouse model in comparison with the control group (Figs. 4 and 5). SD with polymyxin B was also effective in reducing mortality in the pseudomonas-contaminated group but not in the nalidixic acid-treated animals. This is not surprising because Ps. aeruginosa is generally sensitive to polymyxin and not sensitive to nalidixic acid; nalidixic acid may have had only a slight suppressive effect on pseudomonas colonization at the dose it was given.

The conclusion that can be drawn from this experiment is that if the model (with individuals that were not individually treated other than with some form of SD) is representative for the situation that may occur in humans after an A-bomb explosion, then large-scale oral antimicrobial treatment:

1. must not be performed with CR-decreasing antibiotics against which resistance occurs among endemically present potentially pathogenic microbes.

2. should be of an SD-type in order to selectively eliminate all susceptible potentially pathogenic organisms from the gastrointestinal tract thereby preventing infection and limiting spread of resistant organisms by preserving the CR-maintaining (anaerobic) microflora.

3. may reduce mortality if it is of the SD-type.

4. may reduce spread and incidence of infection from enteric pathogens such as Salmonella, Shigella, Yersinia, and Campylobacter.

5. may, with properly selected antimicrobial drugs, also limit the incidence of acute bacterial bronchopneumonias such as staphylococcal and pneumococcal pneumonia as well as Legionnaires' disease.

LARGE-SCALE SD TREATMENT AMONG A-BOMB VICTIMS

Based on the reported experimental and clinical experience, it is reasonable that SD be explored as a possible medical aid in mass casualty treatment. Not only may SD strongly enhance the survival of radiation victims who acquire less than 100% lethal radiation but it may also limit the occurrence of infections caused by pathogenic bacteria.[25]

DISCUSSION

Data are presented ~~in Table 1~~ relevant to the question of whether medical first aid is possible following a nuclear attack. At a distance from a nuclear explosion at which gamma or neutron radiation is at or above the dose level that causes severe bone marrow damage so that death due to thrombo-cytopenia is inevitable, long-term survival will not be possible without advanced medical care. Because medical aid provided by medical doctors will be virtually nonexisting, discussion is realistic only for measures of enhancement of long-term survival among A-bomb victims who were not lethally irradiated, either because they were (partially) shielded and/or were at a greater distance from the hypocenter.

The most realistic conclusions and recommendations regarding first aid can be deduced from what has occurred in reality, i.e., experience in Nagasaki in 1945. It is important that panic be limited. The public should be informed about radiobiology and infection prophylaxis. They should, for example, know that if they find themselves alive after an A-bomb explosion, they have a fair chance to survive the radiation to which they have been exposed. They should

also know that if they are not wounded, they can pull many people in their neighborhood from under wreckage without much danger of acquiring signicant additional radiation. Fire may begin hours after the explosion, particularly in suburbs in which buildings and houses are constructed of wood. In addition to rescuing others, people should collect as many first-aid materials, food, and beverages as they can before the materials are lost due to fire.

In such a situation, it will not be known until much later what the acquired radiation dose may have been at various locations from which refugees come. Radiation and other trauma plus decreased hygienic circumstances among A-bomb refugees who may for some time live in improvised camps can lead to infections by potentially pathogenic bacteria. Therefore, consideration should be given to providing tablets for SD to all victims who can take them. This regimen should be continued for at least 3 weeks. SD may reduce mortality due to infections in those who have received sublethal radiation and/or extensive burn or other trauma. Secondly, SD may reduce the occurrence of transmissible Gram-negative infections such as salmonellosis, shigellosis, pasteurellosis, and cholera. SD will not enhance survival of those who have received lethal radiation doses. They may die within hours due to a central nervous system syndrome, within the first week due to an intestinal syndrome, or else in the second or third week due to severe bone marrow damage (bone marrow syndrome). The majority of these severe radiation victims, however, will not be able to take any oral medication due to severe nausea. This is of practical importance to "first aiders" in the refugee camps. These camps will, like those in 1945 in Japan, be located several kilometers away (a walking distance) from the area in which secondary radioactivity is induced. For example, these camps may be at the distance at which the POW's in Nagasaki found shelter for several weeks after the A-bomb explosion.

Most antibiotics do affect the CR and will therefore enhance the rapid spread of resistant bacteria, the opposite of what is desired. The use of antibiotics other than those recommended for SD, therefore, should be avoided, if not forbidden. The use of CR-decreasing antibiotics might not be contra-indicated, but it may be restricted to use in those hospitals at greater distances from the explosion site and in the hands of experienced doctors.

Tablets for SD may be available in greater quantities only if they are currently being used in our hospitals. In addition to application in infection prophylaxis in oncologic patients, SD appears to find favorable application in the control of Gram-negative infections in hospitals. A sharp reduction of the incidence and the spread of (multi-resistant) Gram-negative infections in intensive care units (in which long-term artificial ventilation is often indicated) and in burn units has been reported recently, in a workshop on CR held in Utrecht.

In this paper I have confined myself to a situation in which nuclear weapons are used on a limited scale, although they may be heavier than bombs used in 1945 in Japan. If nuclear weapons are ever used on a large scale as anticipated by Abrams and Von Kaenel,[25] then circumstances may develop as they describe. However, in that case, it is recommended that survivors of the explosion who can walk should first try to rescue others.

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DISCUSSION PERIOD WITH DR. VAN DER WAAIJ

DR. McCABE: I wonder if Dr. van der Waaij could speciate the anaerobes he feels are responsible for colonization resistance?

DR. VAN DER WAAIJ: This is the key question, and I have no answer other than that this presumably is a Gram-positive fraction susceptible to penicillin or bacitracin. Colonization resistant flora are not susceptible to polymyxin, which will reduce certain Bacteroides species we have studied.

DR. McCABE: Probably not Bacteroides fragilis.

DR. VAN DER WAAIJ: No. It is important to study the regulation of Kupffer cells and macrophages, and also the activity of the bone marrow. We are interested in the possible side effects of decontamination, hoping that we might influence it by eliminating the potential pathogens through the activity of the bone marrow stem cells. In that way we might save a number of healthy stem cells during leukemic chemotherapy.

After approximately 1 week of polymyxin treatment, the average fraction of cells, which is normally on the order of 16% to 18% stem cells in cycle, falls to approximately 4%. Bacitracin-enhancing growth of aerobic Gram-negative bacteria raised the fraction of stem cells in cycle to between 33% and 36%. We are now in the process of investigating what that means in terms of susceptibility to chemotherapy.

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TREATMENT OF GRAM-NEGATIVE BACTEREMIA, ENDOTOXEMIA, AND SHOCK WITH ANTIBODY TO CORE GLYCOLIPID

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INTRODUCTION

The purpose of this report is to describe recent observations on the use of intravenous human globulin against core glycolipid in the treatment of experimental Gram-negative bacteremia and endotoxemia. The development of the gamma globulin is based on previous studies that demonstrated a sharp reduction in mortality rate in patients treated for Gram-negative bacteremia with intravenous antiserum against core glycolipid in the J5 mutant of Escherichia coli. Since concentrated gamma globulin preparations from pooled hyperimmune J5 human antiserum should give even better protection than antiserum, and offer important advantages with respect to storage and administration, we carried out a series of studies on the safety and effectiveness of intravenous J5 human gamma globulin. We used a gamma globulin preparation containing both IgG and IgM because immunoglobulins can protect against endotoxemia, and IgM appears to be necessary for protection against Gram-negative bacteremia. In evaluating the safety of the J5 gamma globulin preparation, we gave special attention to the nonspecific activation of complement, which is considered to be responsible for adverse clinical reactions after intravenous administration of commercial gamma globulins.

BACKGROUND

This research began 15 years ago when Gram-negative bacteria had replaced Gram-positives as the major cause of fatal bacterial infections in patients. Perhaps the main reason for this change is the success of antibiotics against most pathogenic Gram-positive bacteria. By comparison, Gram-negatives are less susceptible to most antibiotics to begin with, and have a remarkable capacity to become resistant to these drugs. The Gram-negatives also have toxic lipopolysaccharides or endotoxins that are not found in the Gram-

positives, and can cause fatal shock. The potential importance of LPS in causing bacteremic shock is obvious from the striking difference in the incidence of shock in the two forms of bacteremia. In an extensive study we found that Gram-negative bacteria are recovered from the blood in 90% of patients with bacteremic shock, whereas shock is unusual in bacteremia caused by staphylococci, pneumococci, and other streppococci.[1] The problem is compounded by the growing numbers of immunosuppressed patients who lose their natural immunity to intestinal Gram-negative bacilli. These three factors (drug resistance, LPS, and immunosuppression) are held primarily responsible for the unremitting high incidence of bacteremias due to Gram-negative organisms and a mortality rate in these infections of 35% to 60%.

In looking for an alternative to antibiotic therapy, we set out to develop an antiserum against the LPS. We found that antisera against smooth LPS prevented all of the toxic reactions induced by endotoxin.[2-6] In order to get around the problem of the antigenic heterogeneity of LPS among the species and serotypes of pathogenic Gram-negative bacteria, we took advantage of the observation that the polysaccharide portion of LPS consists of two regions: (1) the central R core and (2) the side chains carrying the determinants of "O" antigenic specificity.[7-10] Although the structure of "O"-antigen varies widely among Gram-negative bacilli, the structure of the core is similar in all species. This is especially true of the backbone, which contains only heptose, phosphate, ketodoxyoctonate (KDO), and hexosamine. This uniform structure in the core LPS provided a basis for developing one antiserum against all Gram-negative bacteria responsible for serious human infections.

We prepared antiserum against core LPS by immunizing animals with a vaccine made from the rough mutant of E. coli 0111 B4, known as J5 (4). The core of this mutant possesses only "backbone" sugars plus glucose. Although deficient in UDP-glu-4-epimerase, the J5 mutant was at first able to incorporate exogenous galactose and thus attach "O" side chains. From this J5 strain we derived a new mutant that could no longer incorporate galactose from the medium, so that its core became exposed for purposes of immunization.[11] Core antiserum prepared by immunization with a vaccine composed of J5 cells, or purified J5 LPS, produced high titers of antibody to core LPS and a potent

antitoxin against LPS that can neutralize all its toxic properties. The J5 antiserum lowers the mortality rate from endotoxin in experimental animals and gives as good protection against purified smooth LPS as the homologous smooth antisera. The J5 antiserum also prevents the local Shwartzman reaction, disseminated intravascular coagulation (DIC), and renal cortical necrosis.[4,5] Thus, J5 antiserum prevents all toxic manifestations of Gram-negative bacteremia that are attributed to endotoxin.

Antiserum against the J5 mutant was even more successful in the treatment of overwhelming bacteremias produced by Gram-negative bacteria.[11,12] The therapeutic trials with antisera were carried out in a unique model devised in this laboratory for reproducing the syndrome of Gram-negative bacteremia in patients.[13] It is based on our observation that the rabbit normally lacks coliform bacteria in its bowel. The bowel of the rabbit can thus be colonized by feeding any of the Gram-negative bacteria responsible for human septicemic shock, and the animal develops fatal bacteremia if made neutropenic by nitrogen mustard. This model resembles lethal bacteremia in patients with respect to the endogenous source of the bacteremia, the immune disturbances, and the prominence of shock. A slightly different technique was used for Pseudomonas aeruginosa than for othe Gram-negative rods. Lethal Pseudomonas bacteremia was produced by instilling 10^7 bacteria into the conjunctival sac of nitrogen mustard-treated rabbits.[14] When bacteremic rabbits were treated with J5 rabbit antiserum, 40-70% survived, in contrast to a survival rate of less than 10% in controls.[11,12]

We found that J5 antisera will protect against endotoxins or live bacteria belonging to the genera Escherchia, Salmonella, Klebsiella, Serratia, Pseudomonas, and Haemophilus. In addition to treatment of bacteremia with antiserum, we found that active immunization with J5 vaccine or passive prophylaxis with J5 antiserum gave solid protection against subsequent challenge with Pseudomonas in neutropenic rabbits.[12] This protection lasted at least a month, and was as good as that provided by immunization with the homologous Pseudomonas vaccine. It is especially noteworthy that a vaccine composed of E. coli 0111 (the parent strain of the J5 mutant) did not generate protective antibody against Pseudomonas and other heterologous Gram-negative

bacteria. In other words, the protection observed required loss of "O" antigenic chains, so that the core would be exposed to stimulate antibody to this common antigenic region of LPS.

These successful results in experimental animals have been reproduced in patients who were treated with human J5 antiserum. For the past 7 years we have conducted a double-blind trial of human J5 antiserum in over 300 patients with a clinical diagnosis of Gram-negative bacteremia. The human antiserum was obtained from young men who were vaccinated with subcutaneous injections of 5×10^9 killed J5 cells. Among the 304 patients who received 3 ml/kg of either J5 antiserum or preimmune control serum intravenously soon after the onset of illness, a diagnosis of Gram-negative bacteremia was made in 212 (70%). The death rate from Gram-negative bacteremia was lowered from 42/109 (39%) in controls to 23/103 (22%) in those given J5 antiserum ($P = 0.011$). Of those in profound septic shock, 22/39 (56%) of the J5 group recovered from shock, in contrast to 11/38 (29%) among the controls ($P = 0.015$). The control group and the antiserum group were almost identical in age, sex, race, incidence of neutropenia, severity of underlying disease, granulocyte transfusions, and use of high-dose steroids. In view of this ability of antiserum to core LPS to lower the death rate from Gram-negative bacteremia and septic shock, this human antitoxin against LPS should be considered part of the standard treatment of Gram-negative bacteremia.[14]

MATERIALS AND METHODS

J5 globulin was extracted from human J5 antiserum as whole gamma globulin containing IgM, IgG, and IgA fractions. The human J5 antiserum was prepared by immunizing healthy men with J5 vaccine.

J5 vaccine was made from stationary-phase *E. coli* J5 bacterial cells in 18-hour trypticase soy broth cultures. The cells were removed by centrifugation, washed three times in sterile 0.15 M sodium chloride, and boiled for 2.5 hours; the concentration was adjusted spectrophotometrically to 5×10^9 bacteria per millimeter (22% light transmission at 610 μm). Phenol was added to a final concentration of 0.5 g per deciliter, and the vaccine was bottled

in 20-ml quantities. The vaccine was checked for sterility and for safety by testing in mice and guinea pigs, in accordance with the regulations of the Bureau of Biologics. Intravenous immunization of rabbits with the vaccine produced J5 lipopolysaccharide antibody titers of 1:512 to 1:2000 as measured by passive hemagglutination.

Human antiserum was prepared in healthy heterosexual male volunteers, 18 to 35 years old, who were engaged as policemen, firemen, and university students. They were screened for past illnesses, allergies, chronic medication, and given complete physical and laboratory examinations, which included serum hepatitis B antigen tests, hematocrits, VDRL, complete blood counts, urinalysis, and serum aspartate-aminotransferase levels. Then 500 ml of nonimmune blood was collected from each subject before vaccination to provide control serum for nonimmune gamma globulin. Six weeks later, they were immunized subcutaneously with two 1-ml injections of J5 vaccine simultaneously in three separate sites and with two additional 1-ml injections 48 hours later. This schedule was chosen after exploring a number of others because it proved to be the simplest way of quickly inducing high antibody titers. Antiserum was collected at the peak of the antibody response, 2 weeks after the first injections. The mean serum titer against J5 LPS showed a rise over fivefold in vaccinated subjects, with the peak reaching 1:64 from a preimmune peak of 1:8, as measured by passive hemagglutination.

Gamma globulin was prepared by ammonium sulfate fractionation of the proteins in human J5 antiserum. Each unit (500 ml) of blood was collected aseptically, 2 weeks after the first injection of J5 vaccine, into a plastic blood pack without anticoagulant (Fenwal Laboratories, Deerfield, Illinois), allowed to clot at 25°C for 2 hours, and stored at 4°C overnight. The serum was separated, spun free of erythrocytes, and the amount obtained from four donors was pooled. Ammonium sulfate was dissolved by heating 770 g/liter of distilled water. The hot solution was filtered through Whatman No. 1 paper and cooled, and the pH adjusted to 7.2 with concentrated NaOH. Cold saturated

$(NH_4)_2SO_4$ was added drop by drop to an equal volume of serum at 4°C to give 50% $(NH_4)_2SO_4$, dissolved in phosphate-buffered saline (pH 7.0), and brought to one half of the original volume of serum. The precipitation and washing were repeated twice, and the $SO_4^=$ ions were removed by dialysis against PBS. The complete procedure was done at 4°C with sterile nonpyrogenic equipment. An aliquot from each gamma globulin pool was checked for sterility and for freedom from pyrogenicity by intravenous inoculation of 3 ml into each of three rabbits.

Safety of the J5 human gamma globulin was measured by injecting 10.0 ml IV in albino rabbits, 1-2 kg, and observing them for the development of fever and for a drop in serum complement levels. Fever was measured rectally with a thermistat probe at hourly intervals for 3 hours before injection and at 15 min, 30 min, and hourly intervals for 6 hours after. Complement levels were measured in blood drawn from the ear artery 30 min before, and 20 and 40 min after intravenous injection of 10 ml J5 human gamma globulin. The complement levels and pyrogen response were not determined in the same rabbits. Fifty percent hemolytic units of complement were measured by the method of Osler, Strauss, and Mayer. Anticomplementary activity was also determined by the addition of J5 human gamma globulin to fresh normal human serum. Three parts of human gamma globulin were mixed with 7 parts of human serum (i.e., in a proportion in excess of that anticipated in the circulation immediately after injection) and incubated for 1 hour at 37°C. Complement levels were compared in these test samples with those in controls composed of 7 parts of the same human serum mixed with 3 parts sterile normal saline instead of J5 human gamma globulin.

Potency of the human J5 gamma globulin was measured by its ability to protect mice from endotoxemia and neutropenic rabbits from Gram-negative bacteremia. Four groups of 6 mice were each given 1.0 ml J5 human gamma globulin intravenously and challenged 24 hours later with four graded doses of IV endotoxin prepared from E. coli 0111 B4. The LD_{50} in those given J5 human gamma globulin was compared in controls given gamma globulin prepared from the

corresponding nonimmune human sera obtained before vaccination from the same subjects who donated J5 antiserum for the J5 human gamma globulin. Efficacy of J5 human gamma globulin is indicated by significant protection of mice (i.e., rise in LD_{50}) against E. coli endotoxin.

Neutropenia was produced in white New Zealand rabbits by injecting 3 mg/kg nitrogen mustard IV. Seventy-two hours later, when neutropenia was profound, the conjunctival sac was inoculated with 10^7 Pseudomonas aeruginosa. The therapeutic potency was examined by giving 10 ml of a 1% solution of J5 human gamma globulin IV 24 hours after bacterial inoculation when the animals had already developed fever and bacteremia. The mortality rate in these animals was compared to that in controls given IV the same dose of nonimmune gamma globulin prepared from serum obtained before vaccination from donors of J5 gamma globulin. The efficacy of J5 gamma globulin was measured by its ability to prevent deaths in bacteremic rabbits, and the significance of protection of treated over controls was established by the chi square technique.

RESULTS

General Properties of J5 Gamma Globulin (Intravenous)

The product obtained by NH_4SO_4 fractionation produced a clear, slightly opalescent, stable solution in sterile saline (0.85% NaCl) in concentrations up to 16%. The solution was stable for at least 6 weeks of storage (4^oC) with no precipitation or sedimentation of protein. The immunoglobulin solution was sterile and produced no fever in rabbits, when 10.0 ml of 0.1% solution was injected intravenously. Rabbits and mice given 1% solutions of the J5 gamma globulin exhibited no signs of sickness in contrast to those receiving whole human serum. Whole human serum caused weakness, ruffled fur, diarrhea, tachypnea, and transient paresis.

The concentration (mg/dl) of each immunoglobulin was IgG 593, IgM 95, and IgA 121, as measured by rate nephelometry in a Beckman immunochemistry system.

Anticomplementary Activity In Vitro

A 1% solution of J5 gamma globulin, prepared from a pool of four donors, was stored for 5-1/2 weeks at $4^{o}C$ and then examined for anticomplementary activity by mixing 0.3 ml of the gamma globulin with 0.7 ml of fresh human serum. After incubation of the mixture for 1 hour at $37^{o}C$, the complement levels were determined and compared to those in aliquots of the same serum with equal amounts of either 0.85% sterile non-pyrogenic NaCl solution or a commercial preparation of intravenous human immunoglobulin G (Gamimmune, Cutter Biologicals). In all three preparations the complement levels after 1 hour incubation was 56.3 Ch_{50} units/ml, i.e., there was no anticomplementary activity in either gamma globulin preparation. Since J5 gamma globulin had been stored for 5-1/2 weeks, the results indicate that anticomplementary aggregates do not form in storage.

Anticomplementary Activity In Vivo

This was determined in 3-kg albino rabbits by measuring complement levels in blood drawn before and after intravenous injection of 10 ml of a 1% solution of J5 human gamma globulin prepared from a four-donor pool. Before injection of gamma globulin, 5 ml of blood was drawn from the median artery of the rabbit ear, and again at 20 and 40 minutes after the gamma globulin injection. The blood samples were immediately placed on ice to preserve complement levels, and serum was separated after clotting by centrifugation in the cold. Control rabbits received intravenous injections of 10.0 ml of non-pyrogenic sterile physiologic saline (0.85% NaCl solution). Table 1 shows that the J5 gamma globulin was not anticomplementary *in vivo* because the changes in complement levels in the rabbits given J5 gamma globulin were not significantly different from the controls.

TABLE 1. COMPLEMENT LEVELS IN RABBITS AFTER IV INJECTIONS OF 10 ML J5 HUMAN GAMMA GLOBULINS (J5GG)

Rabbit	IV Injection	Time After Injection*	CH_{50}	% Change
1	Saline	0	20.9	
		20	19.6	-6.2
2	Saline	0	20.1	
		20	18.5	-7.97
		40	16.4	-11.2
3	J5GG	0	18.6	
		20	17.7	-4.8
4	J5GG	0	20.1	
		20	18.1	-9.9
		40	16.7	-7.7
5	J5GG	0	19.4	
		20	18.6	-4.1
		40	17.2	-7.5
6	J5GG	0	19.1	
		20	18.6	-2.6

*Minutes

Protective Properties of J5 Gamma Globulin Against Endotoxin

Prevention of death from endotoxin in mice

Twenty-four hours after receiving 1.0 ml J5 human gamma globulin (1%) intravenously, 42-day-old female CF1 mice were challenged with graded doses of E. coli 0111 endotoxin, as indicated in Table 2. Control mice received preimmune gamma globulin taken from the same donors before J5 vaccination. As noted in Table 2, the J5 immune globulin gave marked protection, so that the amount of endotoxin required to kill 50% of the animals rose from 115 µg to 273 µg ($P = < .01$ by chi squares; with Yates correction $P = < .02$).

TABLE 2. PROTECTION OF MICE WITH IV HUMAN J5 GAMMA GLOBULIN AGAINST FATAL ENDOTOXEMIA

Dose of LPS* (μg)	Deaths/Total at 72 Hr: Preimmune Gamma Globulin	J5 Immune Gamma Globulin
500	6/6	6/6
250	7/7	3/7
125	4/7	0/7
62.5	0/6	0/6
Total	17/26	9/26
LD_{50}**	115μg	273μg

* E. coli 0111 lipopolysaccharide
** P = .01; with Yates correction = .02

Prevention of death from Gram-negative bacteremia

Human J5 immune gamma globulin was given intravenously in a dose of 10 ml to each of 14 neutropenic rabbits with bacteremia due to Pseudomonas aeruginosa. A group of 14 control neutropenic rabbits with P. Aeruginosa bacteremia each received 10 ml IV of preimmune gamma globulin. As shown in Table 3, the J5 immune human gamma globulin cut the mortality rate from 86% to 21% (P = .001).

TABLE 3. PROTECTION AGAINST LETHAL PSEUDOMONAS BACTEREMIA IN NEUTROPENIC RABBITS TREATED WITH INTRAVENOUS J5 HUMAN IMMUNE GLOBULIN

Type of Immunoglobulin	HA Titer*	Mortality**
Preimmune	8	12/14 (86%)
J5 Postimmune	32	3/14 (21%)

* Reciprocal of passive hemagglutination titer against erythrocytes sensitized with JF lipopolysaccharide
** P = .001 (Chi square)

DISCUSSION

These studies indicate that intravenous human J5 gamma globulin is effective against endotoxemia and bacteremia, that it is not anticomplementary *in vitro* or *in vivo*, and that it has no side effects in experimental animals. This preparation differs from other preparations of intravenous gamma globulin in its preparation and content. Other preparations have been prepared mainly by alcohol fractionation of pooled human plasma (fraction II), and consists essentially of IgG. Our preparation is prepared by ammonium sulfate fractionation of serum, and contains both IgG and IgM as well as IgA. Since IgG and IgM can each protect against endotoxemia, and since IgM appears to be essential for protection against bacteremia, we chose to prepare a complete immunoglobulin containing both fractions.

In the past, serious untoward reactions (e.g., "anaphylactoid complications) from immunoglobulin preparations have occurred after intravenous injections of products containing IgG and almost exclusively in agamma-globulinemic patients.[15] In patients having no immunoglobulin deficiency, these IgG preparations have been infused intravenously without untoward reactions.[16] Since the J5 human intravenous gamma globulin is intended for treatment of bacteremia in patients who are not deficient in immunoglobulins, it is unlikely that the mechanism responsible for such reactions would operate in recipients of the J5 immunoglobulin. In addition, the presence of IgM along with IgG may also help avoid reactions to IgG alone. At least this was the case in experimental animals given J5 human immunoglobulin intravenously. The freedom from anticomplementary effects both *in vitro* and *in vivo* seem to give some assurance that complement activation in patients may not be a problem. The absence of any discernible untoward reactions in mice or rabbits given large doses of J5 human immunoglobulin was in striking contrast to the marked toxicity of serum or plasma in these animals, which encourages us to anticipate that this gamma globulin preparation will be well tolerated intravenously in patients. If so, it should be a useful product for treating Gram-negative bacteremia, because it is highly effective against experimental endotoxemia and bacteremia.

The main concern in its preparation is the risk of hepatitis. The use of large pools containing immunoglobulins from multiple donors assures adequate levels of protective antibody against hepatitis viruses, so that conventional gamma globulin does not transmit hepatitis. The use of large pools of serum for preparing J5 gamma globulin might provide similar assurance against that risk.

ACKNOWLEDGMENTS

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DISCUSSION PERIOD WITH DR. BRAUDE

DR. SANTOS: Can you tell me how these patients were treated in terms of what antibiotics were used? We have had a number of Gram-negative sepsis cases which we can treat relatively well with antibiotics. I would like to know what were they treated with and how soon?

DR. BRAUDE: I left out the slide showing the appropriateness of the antibiotic therapy. But they are extremely well-masked in this respect. Secondly, it is true that patients who are not deathly ill from Gram-negative bacteremia will do very well with antibiotics.

I would like to emphasize the fact that we picked the cases that looked like they were going to die in spite of antibiotics for this study. We did not take urosepsis, for example, because those patients have an extremely high survival rate.

DR. SANTOS: Presumably they were on the appropriate antibiotic when you tried.

DR. BRAUDE: Yes.

DR. SANTOS: And had been on it and were failing.

DR. BRAUDE: Right.

DR. McCABE: Abe, you choose to term this anti-endotoxic.

DR. BRAUDE: Yes.

DR. McCABE: Yet, as I recall, we both agree that the administration of antisera to the core LPS enhances intravascular clearance of bacteria. If you add it to an in vitro system, several people have shown it opsonizes bacteria. If you use the granulocytopenic model you decrease the frequency of bacteremia. Why then do you say anti-endotoxic rather than antibacterial?

DR. BRAUDE: Mainly because it has much more spectacular effects in blocking the toxic activity than in clearance. Secondly, to emphasize my prejudice and favor of endotoxin being important in the pathogenesis of Gram-negative bacteremia. But you are right, it also has antibacterial properties as well.

DR. MURANO: Could you tell us a little more about the storage period. How long have you looked at this material?

DR. BRAUDE: The studies which I showed today were on preparations that had been stored in the cold for six weeks.

DR. MURANO: And you did not observe any polymerization.

DR. BRAUDE: No.

DR. MURANO: Degradation?

DR. BRAUDE: No.

UNKNOWN: Regarding the issue of IgM versus IgG as the protective antibody in your J5 antiserum, did you ever make an effort to separate IgM and IgG in the rabbit antisera and then quantitatively compare those two antibodies for their ability to protect?

DR. BRAUDE: Yes. We have done this four times to convince ourselves of it. Each time we find that we can get even higher amounts of IgG antibody than we have IgM in a given serum. But the IgM only is protective against bacteremia and the IgG is not.

Now the same is not true of protection against the toxin. We can get antitoxin effects, protection against the Shwartzman reaction with IgG. I was thinking about some of the things Dr. McCabe made me say about why I called it an antitoxin and I would like to supplement those remarks as follows.

The reason why I think we were more impressed with its antitoxic properties is that we had difficulty demonstrating opsonization in vitro with large numbers of different species of organisms, even though we were getting good clearance in vivo. There is no question about the fact that clearance in vivo is a lot more impressive with J5 antibody than opsonization in vitro.

I feel that we can best explain the difference on the basis of the antitoxic properties of J5 antibody, namely by preventing shock and circulatory disturbances, particularly with respect to liver blood flow during endotoxic shock. If we prevent the short circuiting of the liver perhaps the clearance of these organisms by the reticuloendothelial system and the Kupffer cells would be increased.

DR. McCABE: In our experience with the RE mutant we have not been able to show it to be a very strong opsonizing agent *in vitro*. The reports that were done with this were done with dead bacteria and chemoluminescence, not with whether live bacteria were ingested and killed.

Going on to the second point you made, if we do bacterial LD50's with *Klebsiella pneumoniae*, we can increase the LD50 of the numbers of organisms required to kill by 100-fold but we can rarely increase the LD50 of endotoxin by more than four or fivefold.

DR. SANTOS: I am led to believe that a hyperimmune serum in a human would not be as useful as a primary immunization because of the 19S antibody. Ordinarily I would think you would have 19S and 7S. One should consider that a vaccine may be better. If they had been immunized and you gave them a booster, maybe most of this is 7S. Could you comment?

DR. BRAUDE: I think very little is to be gained with a booster.

AD-P003 882

EXPERIMENTAL PSEUDOMONAS AERUGINOSA BURN INFECTIONS

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INTRODUCTION

Despite major advances in medicine and biotechnology, about 12,000 persons within the civilian community of the United States die annually from thermal burns, while about 300,000 suffer severe burns.[1] The incidence and the severity of burns sustained in combat situations have increased significantly since World War II as the result of the development and changes in weapon systems used.[1] For example, during World War II, 0.7 percent of casualties in the Russian Army was due to burns; in 1965, burn casualties reached 2 per cent in the North Vietnamese Army and later rose to 12 percent. In the 1973 Israeli-Egyptian conflict, the burn casualty rate for Israeli tank personnel was 9.3 percent. In addition to the trauma caused by the burn itself, a severe burn injury causes suppression in the function of the victim's immunological defenses so that patients are extremely susceptible to bacterial and fungal infections.[2] One of the organisms most frequently encountered by burn victims is Pseudomonas aeruginosa. During the recent Middle East conflict in Lebanon (1982), it was estimated that about 15 percent of the casualties in the Israeli Army were burn victims and that the most common infection encountered was caused by P. aeruginosa (I. Brook, personal communication).

The difficulty and ethical problems involved in studying patients with life-threatening infections has made it necessary to conduct evaluations of therapeutic approaches and the course of infections in a number of animal models. These models deal with particular diseases, microorganisms, or

aspects of a given organism. Frequently, however, the trauma of the experimental burn or mechanical injury contributes significantly to the death of the animal, thus making difficult the evaluation of the parameters being studied difficult.[4] In this report, we describe a nonlethal burn in the mouse model,[3-6] which mimics the human clinical situation, and has been used extensively in studying P. aeruginosa virulence factors[5,7-13] and various experimental P. aeruginosa vaccine preparations.[5,13-15]

EXPERIMENTAL BURN TRAUMA

Common laboratory animals are highly resistant to P. aeruginosa infections. To overcome this resistance and successfully initiate an experimental infection, investigators have taken a variety of special steps. These include use of unrealistically high doses of bacteria, addition of virulence-enhancing factors such as mucin to the inoculum, or pretreatment of experimental animals with cytostatic drugs such as cyclophosphamide. It is obvious, of course, that such models do not fully reflect the clinical situation and are of minimum clinical relevance. However, the burned mouse model we have used overcomes these problems.

In our model, the backs of mice (18-20 g) of either sex were shaved, and the mice were anesthetized with methoxyflurane or similar anesthetics such as halothane or enflurane. Under the conditions used in our laboratory, we found that halothane was associated with a higher mortality than methoxyflurane and that mice anesthetized with enflurane had a tendency to recover during the trauma. A template with a 2.5 by 2.5 cm opening (corresponding approximately to 15 per- cent of the total body surface) was then placed on the shaved area of the anesthetized mouse, covered with 0.5 ml 95-percent ethanol, and ignited for 10 sec. Immediately following the trauma, 0.5 ml of a bacterial suspension, at the desired concentration, was injected subcutaneously into the burned area. Control animals received an equivalent volume of buffered saline. The burn injury alone was not lethal. The control non-infected mice recovered from the trauma within 1 to 2 hours.

HISTOPATHOLOGY

The white blood cell (WBC) count of the traumatized non-infected mice did not differ significantly from that seen in the normal untreated mice (Table 1)[17]. Traumatized infected mice (1 LD_{50}), between 22-28 hr post-infection, had a significant decrease in the WBC count. At 48 hr post-infection, most of the surviving mice recovered normal WBC count; few mice remained leukopenic up to 72 hr post-infection.

TABLE 1. LEUKOCYTE LEVELS IN BURNED INFECTED MICE[a]

HOURS POST-INFECTION	LEUKOCYTE COUNT ± SD ($10^3/mm^3$)	p[b]
Control-not traumatized	5.3 ± 1.4	
3	5.7 ± 1.8	0.05
6	7.4 ± 1.9	0.05 - 0.02
12	5.0 ± 1.0	0.05
22	2.1 ± 0.8	0.001
28	1.7 ± 0.8	0.001
48	4.8 ± 1.8	0.05
72	4.5 ± 1.8	0.05,

[a] Wretlind and Pavlovskis (1983)

[b] Student's t test, 6-8 mice per group

Histologic examination of skin sections from the burn area at various times post-trauma showed that the trauma was consistent with a full thickness or a third-degree burn injury (Fig. 1).[6] Changes noted included increased basophilia of epidermal cells, marked pyknosis of nuclei, and sloughing of the epidermis. The dermal collagen bundles had a homogenous coagulated appearance instead of their normal fibrillar character. An abrupt transition from necrotic to normal viable skin was usually seen at the periphery of the thermal injury. At 48 hr post-trauma, acute inflammatory infiltrates were present in both subcutaneous and deep dermal areas. This was more pronounced in the infected mice than the traumatized non-infected mice. No histologic differences were seen in abdominal organs taken from normal untreated mice and compared to those taken from traumatized non-infected mice.[5-6]

EXPERIMENTAL INFECTIONS

The LD_{50} of both normal infected mice and traumatized infected mice are given in Table 2.[4,5] A significant increase in susceptibility to P. aeruginosa is seen in traumatized mice as compared to the untreated mice. At least a three log_{10} difference was demonstrated. With some strains of Pseudomonas, less than 10 organisms were required to initiate an infection. This increased susceptibility of burned mice to P. aeruginosa appears to be specific for Pseudomonas since it was not seen when other bacteria were used to infect the mice (Table 2).[4] The increased susceptibility seen immediately after burning decreases between 8-18 hr post-trauma and does not appear to be dependent on the route of infection.[4] After 20 hr post-infection, P. aeruginosa was isolated from every organ examined (kidney, liver, spleen, lung, heart) as well as blood.[4-6]

TABLE 2. THE MEAN LETHAL DOSE OF NORMAL AND BURNED MICE INJFECTED SUBCUTANEOUSLY WITH BACTERIA

ORGANISMS	REFERENCE	MOUSE STRAIN	MEAN LETHAL DOSE (CFU)[a] LOG_{10}	
			NORMAL MICE	TRAUMATIZED MICE
P. aeruginosa (PA 86)	5	NIH/NMRI CV	6.40	3.36
P. aeruginosa (PA 103)	5	NIH/NMRI CV	6.26	3.08
P. aeruginosa (PA 220)	5	NIH/NMRI CV	4.15	1.00
P. aeruginosa (M-2)	4	CF1	6.11	1.00
P. aeruginosa (UM)	4	CF1	6.04	1.00
E. coli	4	CF1	8.18	7.18
Klebsiella species	4	CF1	7.96	8.08
S. aureus	4	CF1	8.26	8.26
C. albicans	4	CF1	6.67	6.67

[a]Colony-forming units

A similar model using the laboratory rat has been developed by Walker et al.[18] Instead of using an alcohol flame, these authors placed the anesthetized rat in a fixed-area shield with a 5 cm X 7 cm opening (approximately 20 percent of the total body surface), and the shaved dorsum was immersed in a boiling water bath for 10 sec. The trauma resulted in a full-thickness burn and histologic changes similar to those seen in the burned mouse.[2] The increased susceptibility to lethal infection, as in our model, decreased after 8 hr post-trauma.[19]

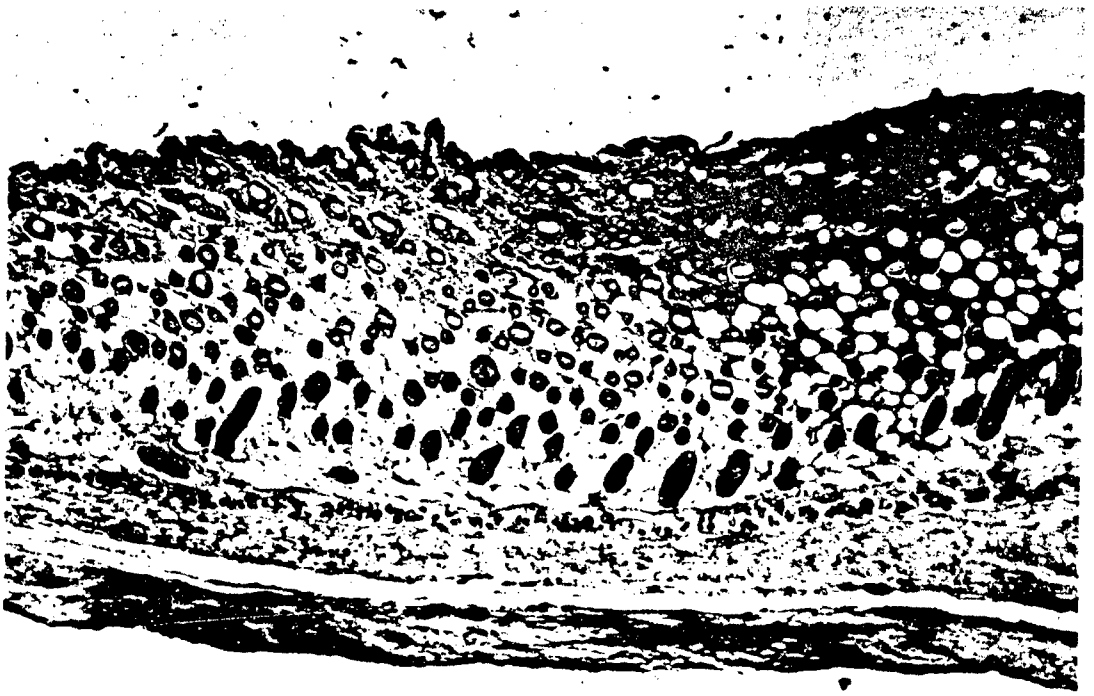


Fig. 1: Photomicrograph of murine skin section from burned, non-infected area at 24 hrs. Note the full-thickness burn (right) with loss of epidermis and coagulation necrosis throughout the underlying area. Scattered neutrophils have begun to enter the deep layers of tissue.

Both the mouse and the rat model appear suitable to study (with minimal discomfort to the experimental animal) the role of various P. aeruginosa factors in virulence as well as to evaluate the usefulness of vaccines, antibiotics, and other means of prophylaxis and treatment of pseudomonas infections.

PROPHYLAXIS OF EXPERIMENTAL BURN INFECTIONS

Previous studies have shown that P. aeruginosa exotoxin A is an important virulence factor in pseudomonas infections. We have examined the efficacy of several exotoxin A toxoid preparations as well as passive protection with

mouse monoclonal antibodies against experimental P. aeruginosa infections in the burned mouse model. Exotoxin A toxoids were prepared by either formalin or glutaraldehyde treatment.[14,15] Mice were immunized with the different toxoid preparations together with the synthetic adjuvant N-acetylmuramyl-L-alanyl-D-isoglutamine. Immunization with three or four doses of the formalin toxoid resulted in a significant rise in antitoxin titers. A significant increase in survival time and rate (50 to 80 percent of the animals survived

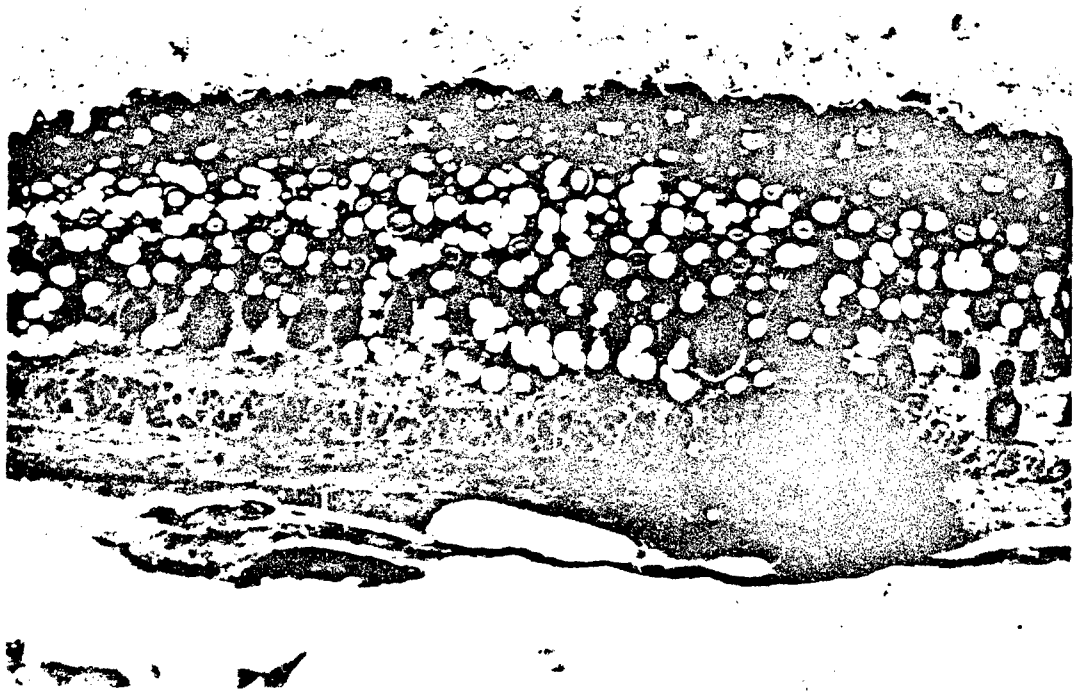


Fig. 2: Photomicrograph of murine skin section from burned, infected area at 48 hrs. Note the full-thickness burn with marked coagulation necrosis and loss of epidermis with extensive infiltration of neutrophils in subcutaneous and deep dermal areas (lower right).

permanently) of these immunized mice was observed following infection with P. aeruginosa. Virtually 100 percent survival was obtained when preinfection immunization was combined with a single dose of gentamicin treatment (Fig. 3). In addition, the immunized mice required a higher infective dose than control groups.[15]

We have also examined the protective effect of intraperitoneally administered mouse monoclonal antibodies against exotoxin A.[20] Preliminary results with one of the three antibody preparations tested indicated that the

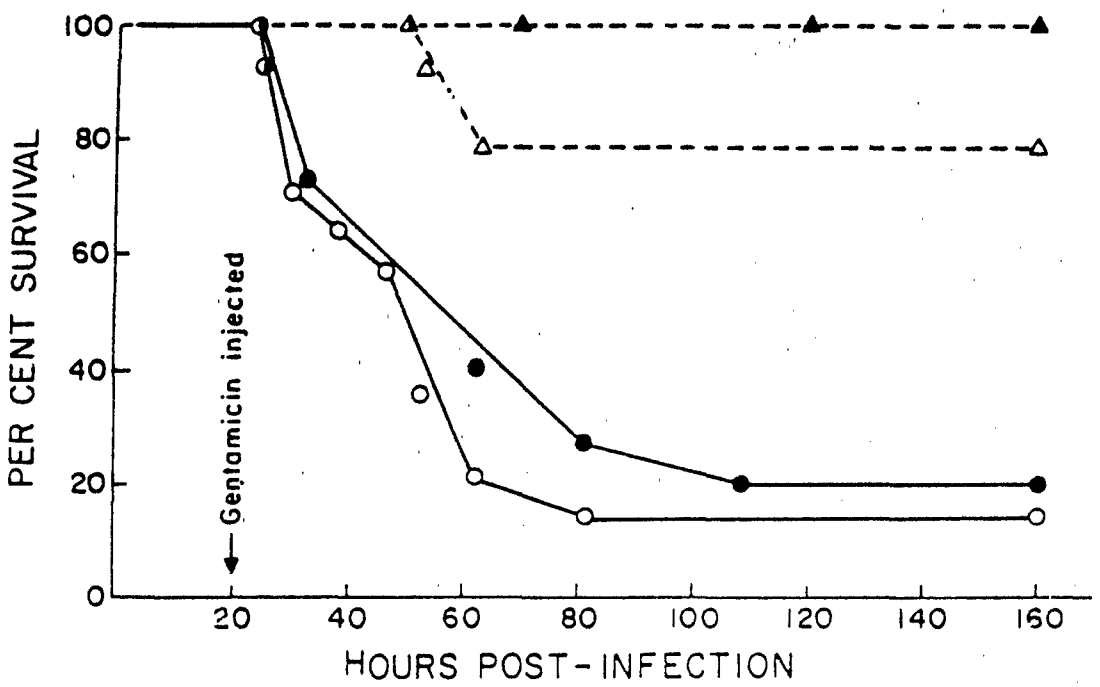


Fig. 3: Survival of burned, infected mice immunized with formalin toxoid (---) or formalin-treated bovine serum albumin (——) and treated with gentamicin (▲, o) or not treated (△, o). From Pavlovskis et al. (1981), reprinted by permission of the American Society for Microbiology.

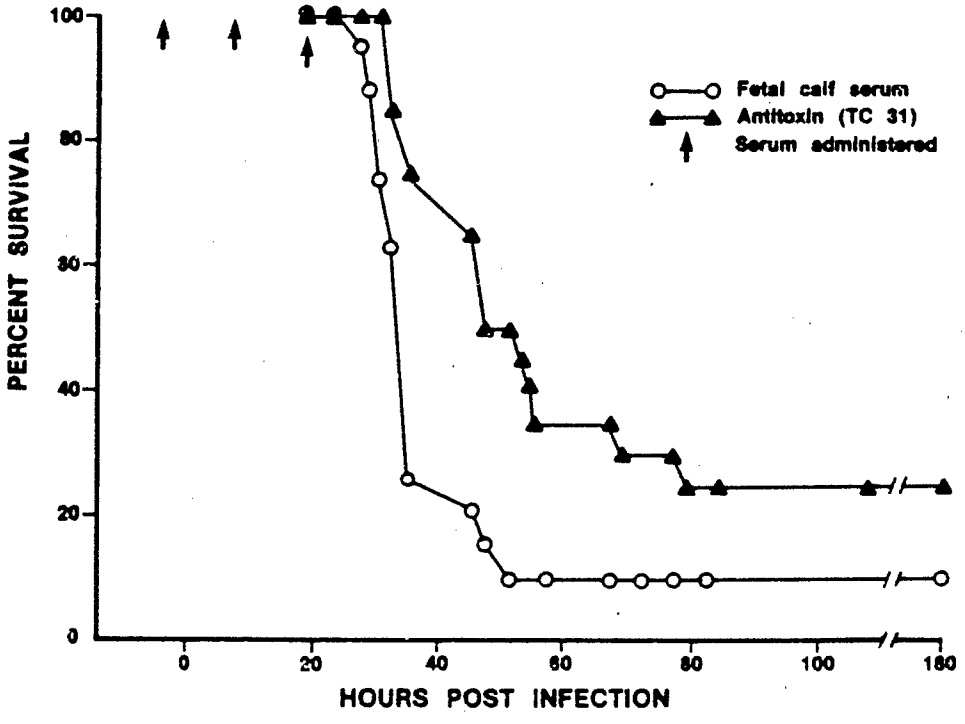


Fig. 4: Survival of burned, infected mice treated with mouse monoclonal antibodies (antitoxin) (Δ) or fetal calf serum (o).

survival time and the number of mice surviving the infection were increased in the group treated with the monoclonal antibody (Fig. 4). Such passive protection may be particularly important in patients who have not been previously immunized or who have lost their immunity as a consequence of trauma.

Most experimental models used to study pseudomonas pathogenicity are inadequate substitutes for clinical infections, since unrealistically large doses of organisms, virulence-enhancing factors, or cytostatic drugs must be

used to initiate and/or maintain the infection. Moreover, severely traumatized animals are difficult to use since the injury often contributes to their death. In the model we have used, a burn is inflicted and infection is reproducibly produced by a small inoculum of bacteria. Another important factor that should be considered in selecting the proper model is the length of the infection. In our experiments with untreated infected animals, septicemia occurred around 20 hr post-infection and death resulted in 40 to 72 hr. This allowed sufficient time to evaluate various treatments and their effect on the course of the infection. Finally, by changing either the size of the trauma or the time between injury and infection, the infective dose or the survival time can be changed to meet the requirements of a given experiment.

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DISCUSSION PERIOD WITH DR. PAVLOVSKIS

UNKNOWN: How does type-specific immunization compare with immunization against toxin?

DR. PAVLOVSKIS: Survival, following immunization with rabbit antitoxin, appears to be dependent on the products produced by the organism rather than the serotype. For example, strain 220, which produces both toxin and large amounts of protease, gave the poorest results. Although the antitoxin neutralized the toxin, the protease was causing other effects. In other words, if an organism produces excessive amounts of protease, we usually do not obtain good protection with polyclonal antitoxin. This suggests, of course, that there are other virulence factors involved besides exotoxin.

Similar results are obtained when mice are immunized with toxoid. The degree of protection depends on the size of the inoculum and the products of the organism. We have not noticed any differences in survival with respect to different serotypes of the organism.

AD-P003 883

PHARMACOLOGIC MANAGEMENT OF SEPSIS

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INTRODUCTION

Systemic sepsis continues to be the most difficult management problem in caring for the combat casualty. The complications of sepsis pervades all areas of injury to the man in the field, whether it is mechanical (missiles), thermal (burns), chemical, biological, or radiation injury. With the advent of tactical nuclear weapons, the problem of sepsis will be much higher in future wars than has previously been experienced throughout the world.

In Vietnam, 50% of the deaths that occurred after the patient arrived at the hospital were related to head injuries.[1] The next largest group of deaths occurred as complications of hypovolemic shock. That is, these patients arrived at the hospital alive; they were resuscitated and surgically treated, but they then died with multiple organ failure. Of those that died, 38% were attributed to hypovolemic shock. Severe systemic sepsis was the cause of death in only 11% of patients who arrived alive at the hospital. One could state that 11% is not a large group of patients; however, this may be the group with the greatest potential of significantly improving. The logic is as follows: (a) Patients arrive with severe head injuries. They are surgically managed, but 50% of the deaths that occur usually are related to the degree of brain injuries. (b) For casualties who die from hemorrhagic shock, most of the available resources are relegated to the care of these patients. Unfortunately, patients in this group have severe blood loss and significant tissue hypoxia; upon arrival, they can be resuscitated and surgically treated, but they have a downhill course, with multiple organ failure. (c) A subset of this population are those who [illegible] sepsis. Unless the patients are adequately cared for with appropriate antibiotics to sterilize the bloodstream and aggressive surgical treatment to debride necrotic tissue and drain abscesses, the patients die. Sepsis is important not only in the military,

but also in the civilian trauma and intensive care units, where it continues to be an increasing problem. The incidence of sepsis, even in the best of hospitals, is 1% in all acute hospital admissions. In the U.S. alone, there are approximately 200,000-400,000 septic deaths annually.

What is the problem? A major impact in sepsis is the overuse or abuse of antibiotics. Bacteria have a biochemical laboratory that outpaces the development of new antibiotics by pharmaceutical producers. Antibiotics for specific short-term use is critical; however, for prolonged sepsis, they may be ineffective. The technological advances in the early diagnosis of abscesses by computerized axial tomography (CAT SCAN) and ultrasound improve the accuracy of the diagnosis of localized infection, and provide the clinician a direction of treatment much earlier in the course than was available 10 years ago. Obviously, the answer to these patients is not simple, and will require laboratory and clinical research to approach the problem.

The purpose of this manuscript is (a) to review the data suggesting pharmacological agents that may benefit the septic patient and (b) to emphasize the adjunctive therapies that should be explored in clinical trials.

HISTORICAL PERSPECTIVE

Sepsis and septic shock have been identified clinically for four decades.[1] Patients are frequently categorized by circulatory state, either hyperdynamic or hypodynamic. Patients in the hyperdynamic state appear to have the most favorable prognosis. The clinical characterization of the septic patient is becoming more refined; however, the complexity of the data makes it difficult to know how best to manage the patient. The diffuse effects of sepsis suggest that many homeostatic mechanisms are out of control, therefore precluding meaningful information from current studies.

Circulatory dysfunction is an early sign in sepsis, and many vasoactive agents appear to participate. These include histamine,[2,3] 5-hydroxytryptamine,[4] angiotensin,[5] epinephrine and norepinephrine,[6] C5a,[7] endorphins,[8]

endorphins,[8] prostaglandins,[9] and kinins.[10] Attempts have been made to utilize antagonists to these vasoactive agents; however, many of the results indicate only transient benefits or even exacerbations of the injury. Antihistamines, alpha and beta blockers, and serotonin inhibitors have not demonstrated sufficiently improved benefits to merit further elaboration. However, the putative role of the prostaglandins, the endorphins, and complement are undergoing intensive investigation, and should be discussed. The inhibitors for these humoral mediators may provide adjunctive therapy in treatment of severe systemic sepsis.

PROSTAGLANDINS

The arachidonic acid-prostaglandin system consists of potent biologically active fatty acids that participate in inflammation, burns, hypertension, peptic ulcer disease, diarrhea, vasomotor regulation, platelet function, gynecological disorders, allergic reactions, fever, and shock-like states.[11] The discovery that non-steroidal anti-inflammatory drugs (NSAID) inhibit prostaglandin synthesis[12] set the stage for credible scientific endeavors in this field.

Northover and Subramanian were the first to utilize NSAID in experimental shock states. The initial studies were done on circulatory function and survival.[13] Plasma prostaglandin levels are increased in many species that have been subjected to endotoxemia, including the subhuman primate.[9,14-20] Early studies were done by assaying the prostaglandins at arbitrary timed intervals rather than at times in which circulatory events were changing. In addition, the similarity in the stress from one laboratory to another was unknown. Following the early studies, the relationship of prostaglandins to the pathophysiology of endotoxin shock was not clear.

Since these initial studies, a number of investigations have shown that shortly after the injection of endotoxin (LD50 concentrations), plasma prostaglandin concentrations increase in mixed venous and arterial blood.[8,16] These studies indicate the association between the mexed venous prostaglandin levels and the increase in pulmonary artery pressures.[14,17,18] In contrast to the

early studies that demonstrated that the PGE's and PGF's were the important prostaglandins released, the present understanding is that while the PGE's and PGF's are released, thromboxane and prostacyclin constitute the largest percentage of end products of arachidonic acid metabolites and are more likely to participate in the pathophysiological events in endotoxemia and sepsis than the PGE's and PGF's.[20-26]

Thromboxane and prostacyclin have received the most interest recently; therefore, additional information is worthwhile, especially because specific thromboxane synthesis inhibitors are now being utilized. Bult et al. were first to report that prostacyclin was present in endotoxemia.[20] Harris and co-workers showed a similar time course of an early increase in thromboxane and a more prolonged rise in primate endotoxemia.[25] In rat endotoxemia, Cook et al. demonstrated early and late increases in both thromboxane and prostacyclin,[23,26] and reported that a non-specific thromboxane inhibitor, imidazole, does improve the survival. Casey et al., in a subhuman primate endotoxin model, clearly demonstrated for the first time that a specific thromboxane synthetase inhibitor prevents endotoxin-induced pulmonary artery hypertension and effectively inhibits thromboxane production, but fails to improve survival.[22] In Gram-negative sepsis, thromboxane and prostacyclin are increased similar to those seen in endotoxemia. In these studies, imidazole did not improve survival, even though it inhibited thromboxane synthesis.[27] These findings indicate that while thromboxane and prostacyclin participate in the pathophysiology of endotoxemia and/or sepsis, the specific inhibition of thromboxane does not improve the survival in either type of shock. The role of prostacyclin in these entities is yet to be determined. Interestingly, prostacyclin infusions have demonstrated improved survival in some forms of shock.[27-29]

The natural question is, "Does endotoxemia represent sepsis or septicemia?" Live E. coli infusions in baboons produce significant increases in plasma prostaglandin levels.[30] Animals with bacterial peritonitis show increased plasma values of prostaglandins about the time blood cultures are positive for E. coli bacteria.[25] Indeed, during Gram-negative sepsis in man, plasma prostaglandin levels are increased.[31,32] Other studies also document

in man that plasma prostaglandin values are increased and participate in the pathophysiology of sepsis.[21]

If the prostaglandins are present, what are their effects in endotoxemia, endotoxin, and septic shock? Non-steroidal anti-inflammatory drugs have been utilized to elucidate the pathophysiological role that the prostaglandins may have in sepsis. First, initial studies utilized pharmacological rather than therapeutic concentrations of NSAID as pre-treatment. Unfortunately, there were great differences between studies in doses and frequencies of NSAID administered.[33-36] Pre-treatment with NSAID, however, showed a significant increase in survival and improved circulatory function, whereas post-treatment with NSAID in overwhelmingly lethal models failed to improve survival.[34,36] Subsequently, studies were completed in dogs and baboons in endotoxin shock that demonstrated that NSAID, even when administered after shock occurred, significantly improved survival.[37] NSAID pre-treatment of animals subjected to Gram-negative peritonitis (without antibiotics) increased survival. When antibiotics were administered in addition to the NSAID, survival was greater than with antibiotics alone.[37] In addition to Gram-negative sepsis, NSAID are reported to improve survival in a Group B streptoccocal rat sepsis model.[38]

Several prostaglandin effects have been implicated in endotoxemia or sepsis: Prostaglandins (a) increase pulmonary artery pressure and decrease systemic arterial pressure,[9,14-20] (b) exaggerate the metabolic derangements,[35,36,39-41] (c) enhance pulmonary dysfunction,[19] and (d) participate in the inflammatory response and in vascular permeability.[42,43] The prostaglandins do not appear to alter the kinin system,[10] serotonin or histamine effects[41,44] or lysosomal enzyme release in endotoxemia.[17] The inhibition of prostaglandins does not alter the coagulation derangements,[17,40] the complement activation,[17] or the leukopenia or thrombocytopenia present in endotoxemia or sepsis.[40] The documented benefits of prostaglandin inhibition in sepsis are the stabilization of cell membranes and the attenuation of circulatory dysfunction. The exact mechanisms by which the prostaglandins participate in the pathophysiology of sepsis are still unknown; however, with the development of specific inhibitors to other enzymes in the arachidonic

acid cascade, more sophisticated and specific information will be forthcoming. It is likely that the prostaglandins operate in the hormonal and neurochemical milieu that homeostatically controls vascular smooth-muscle responses.

Inhibition of prostaglandins in sepsis demonstrates that (a) prostaglandins participate in sepsis in animals and man through as-yet-undefined mechanisms, (b) prostaglandin synthetase inhibitors (NSAID) improve the survival in several animal models of sepsis even administered when blood cultures are positive or after shock has occurred, (c) circulatory function is improved with NSAID, and (d) there is a large scientific data base that supports the use of these drugs in the study of carefully controlled clinical trials.

ENDOGENOUS OPIATES (ENDORPHINE)

Over the past few years, evidence has accumulated that suggests that the endorphine participate in several shock-like states, including endotoxemia, acute hemorrhage, and spinal cord transection.[44] Faden and Holaday first postulated this hypothesis utilizing hypovolemic shock in rats.[45] Others have confirmed the original studies and extended the beneficial effects of opiate antagonists to other species, including man.[8,46-49] The site of action of the endorphin antagonist is unknown, but central and peripheral areas have been investigated.[48,49]

Beta endorphins, one of the major endogenous opiates, are secreted from the pituitary gland, and has been implicated as a cardiovascular depressant.[50] Investigators are actively working to identify the dose-response relationship in animal models to the parameters in sepsis and to evaluate the effects of naloxone in patients in shock-like states.

It is certain that endorphins participate in stress states; however, the future use of the opiate antagonists is yet to be determined. The basic information is accumulating to suggest that these chemicals may be important in the pathophysiology of sepsis.

OTHER AREAS OF PHARMACOLOGICAL INTEREST IN SEPSIS

CALCIUM CHANNEL BLOCKERS

Intracellular calcium overload participates in tissue ischemia.[51] The concept is that as ischemia progresses, acidosis increases, and membraneous portions of the cell disrupt, allowing for a direct extracellular source for calcium overload. It has been postulated that intracellular calcium overload is a final common pathway for ischemia injury[52] and therefore may be important in the irreversible shock-like state. As yet no broad data base has been established for the use of these agents in sepsis, but the ubiquitous nature of calcium portends a therapeutic use of calcium entry blockers in the future.

ANTIBIOTICS

The concept that antibiotics may have a pharmacologic effect independent of their bactericidal activity has been recognized only recently. Interestingly, most of the observations have been with aminoglycosides. These antibiotics influence peripheral cardiovascular control mechanisms, which may include the inhibition of autonomic neurotransmission, vasodilation, reduction in heart rate, and lowering of myocardial contractile state. Their effects reflect reversible interactions of these drugs with excitable membranes.[53] A recent review states that these antibiotics affect transmembrane influx of calcium and that they may be similar to the calcium channel blockers mentioned above. The authors believe that membrane calcium stores are reduced, and therefore less calcium is available for intracellular distribution.[54] The oto- and nephotoxicity of these drugs could be related to a calcium antagonist action.[55,56]

The pharmacological effects of antibiotics may be harmful as well as beneficial in the very ill patient. Consideration of these effects will be required once this fascinating new area is explored.

Numerous other pharmacological agents with potential therapeutic value include local anesthetics, especially lidocaine, opsonic proteins, ATP-$MgCl_2$, and hypertonic crystalloid solutions. Lidocaine improves survival in dog and

baboon endotoxin shock.[39,57] Fibronectin enhances bacterial killing in animals and man.[58] ATP-$MgCl_2$ solutions restore energy reserves in hypoxic states and could potentially protect the cell from further damage.[59] Hypertonic crystalloid solutions may have pharmacological benefits in sepsis that are yet unknown.

CONCLUSIONS

The pharmacological management of sepsis remains controversial. Most of the drugs utilized clinically treat the symptoms of the disease, and are not necessarily directed at fundamental mechanisms that are known to be present in sepsis. A broad data base is emerging, indicating that NSAID should be used in human clinical trials. Prostaglandins are sensitive indicators of cellular injury and may be a mediator for a number of vasoactive chemicals. Opiate antagonists and calcium channel blockers require more in-depth data; however, recent studies generate excitement for their potential use in the critically ill patient. Pharmacologic effects of antibiotics, in concert with other drugs, suggest an entirely new approach to the pharmacologic treatment in sepsis. There is no doubt that new treatment modalities or adjunctive therapies must be utilized to alter the poor prognosis of severe sepsis that we have observed in the past 4 decades.

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DISCUSSION PERIOD WITH DR. FLETCHER

DR. KAPLAN: Have there been any studies in humans as to whether or not these same anti-inflammatories are effective in humans and, if so, in what dose and administration? For instance, the only form of Indocin that I know is oral. Can it be given systemically? I assume that it can. Is it available?

DR. FLETCHER: First of all, there have been no human studies that I know of that have been done with sepsis per se. There have been a number of reported studies that have been done, along lines of immunoregulation or immunosuppression, but not in sepsis per se.

You are quite correct that the nonsteroidal anti-inflammatory drugs are not available for intravenous use at the present time, except for ibuprophen, or Motrin as most of us know it, which is being utilized in clinical studies on myocardial infarction right now.

I do think that it is time that clinical sepsis studies be done with nonsteroidal anti-inflammatory drugs.

DR. SANTOS: Have you studied ARDS in relation to this?

DR. FLETCHER: No sir, we have not.

DR. SANTOS: Has anybody?

DR. FLETCHER: Yes sir. Studies done at Yale demonstrate that early in acute events prostaglandins are elevated. They participate in both pulmonary vascular events and vascular permeability. There have not been any studies in patients though. There does appear to be a dose response relationship to ARDS and the arachidonic acid prostaglandin system.

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BATTLEFIELD WOUND INFECTION

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INTRODUCTION

With the advent of antibiotics, many people felt that wound infections were no longer going to be a serious problem in the battle casualty. First, I hope to dispel the perception that wound infections are not a major problem. Second, I will provide my perception of the most appropriate means of preventing wound infection. The answer to the prevention of wound infection in combat casualties is debridement.

I will try to illustrate the lack of advancement in the prevention of wound infection. Observations were made from 1942 through 1943 in five major hospitals in the U.S. to evaluate the efficacy of sulfonamides in the prevention of wound infection.[1] The prophylactic use of oral sulfadiazine and the local application of a mixture of sulfadiazine and sulfanilamide did not decrease the incidence or severity of local infection or eliminate pathogenic organisms. The occurrence of generalized invasive infection was diminished in the treated group. There was a 6.4% incidence of infection in soft-tissue wounds, twice that in compound fractures, and a fourfold increase in burns.[1]

During the next 10 years, other antibiotics were introduced into clinical practice in addition to sulfonamides. Among these were penicillin, tetracycline, streptomycin, and chloramphenicol. These antibiotics were available during the Korean conflict. The prevalence of wound infection was 26.5%. It was standard practice in Korea that as soon as an individual reached medical attention, he received an injection of 600,000 units of penicillin. Despite treatment with penicillin, the prevalence of wound infection was exactly as seen in 1942-43. There was no significant decrease in infection. There are, unfortunately, no comparable data available from the experience in the Vietnam War.

The next conflict in which reliable data are available is the 1973 Yom Kippur War in the Middle East. The results from two studies will be presented. The first concerned a study of 624 patients.[3] Infection at the time of admission was 9%, and another 5% developed hospital-acquired infection for an overall incidence of infection of 14%. During this particular conflict it was standard practice in the Israeli Defense Force to administer penicillin on the battlefield. All of these casualties received penicillin on the battlefield. Despite over 35 available antimicrobial agents (with the exception of third-generation cephalosporins), the incidence of wound infection had not significantly decreased. In fact, in every one of these conflicts, if you survived your initial wounding, the most frequent cause of death after initial resuscitation was infection. That was true in World War II, the Korean conflict, Vietnam, and the Yom Kippur War.

The British experience during Operation Corporate in the Falkland Islands is the most recent experience. Data were obtained on 658 battle casualties who had been treated at Ajax Bay.[4] The British used a standard protocol in the Falklands: all wounded casualties received a tetanus booster, in addition to one ampule of a combination of ampicillin and penicillin as soon as possible after injury. All wounds were debrided, and were not closed primarily. Casualties with abdominal wounds received intravenously administered gentamicin and metronidazole; individuals with head trauma received sulfadimidine. The result of this regimen was only one case of wound infection at Ajax Bay. The standard holding time was limited. One would not expect to see very much in the way of infection since these individuals were evacuated first to the hospital ship S.S. Uganda and then back to England.[5,6] The data on the actual incidence of wound infection in British casualties in the Falklands are not yet available. British surgeons in the U.K. have indicated that infection did occur. The point is that, despite the fact that multiple antimicrobial agents are available, wound infection is a problem that has not been solved.

FACTORS RESPONSIBLE FOR WOUND INFECTION

What are the factors that predispose the casualty to wound infection? Consideration of these factors provides a basis on which one can appropriately manage wounds. The first, and perhaps the most important factor in predisposing a wound to infection, is the presence of foreign bodies in the wound. Drs. Altemeier and Furste performed a classic study showing the significant increase in wound infection in crushed muscle with foreign bodies.[7] Spores of Clostridium perfringens were injected into guinea pigs. The results were gas gangrene (clostridial myonecrosis). If spores were injected alone, it took 10^6 spores to be able to produce the wound infections. If, on the other hand, the muscle was crushed and then the spores introduced, gas gangrene could be produced with 10^3 spores. If sterile dirt was added to the spores plus the crushed muscle, it took one spore to obtain the same result. The presence of foreign bodies resulted in a million-fold increase in the susceptibility of a wound to infection. The principle of debridement is to get rid of crushed muscle as well as foreign bodies, all of the debris that contaminates combat wounds. The following is another example that will illustrate this point. The gluteas maximus of a rabbit was cut, incised, then crushed with Kocher forceps; finally, nonsterile dirt was added to the wound. This resulted in clostridial myonecrosis in 100% of the animals over a 7-day period.[8]

A study reported by Drs. Elek and Conen from the U.K. illustrates this effect in humans.[9] The objective of this study was to determine the influence of suture material or foreign bodies in the wound on the susceptibility to infection in man. Staphylococci were injected intradermally into the forearm skin of volunteers in an effort to determine the minimal pyogenic dose, that is, the minimal number of staphylococci that would produce a small pustule in the skin. The result indicated that it took between 1 and 5 x 10^6 staphylococci to produce a small pustule. If you put the organism directly on a sterile silk suture and through the skin, the dose of organisms decreased from 1-5 x 10^6 to a range of 10^2 to 10^4. The presence of silk sutures caused a 10^3 - to 10^4-fold increase in susceptibility to Staphylococcus. These data illustrate the importance of foreign bodies in increasing susceptibility to infection. On the practical side, it is a good illustration of the importance

of the good suture technique of not leaving long rabbit ears on the knot. A practical note is in order concerning the use of a bovie. Although a bovie is a very rapid way to produce hemostasis, it may leave devitalized tissue in the wound.

The second factor that is important in wound infections is time lag, the period of elapsed time between injury and treatment. In any discussion of combined injury, time lag becomes of great importance. An excellent example of this occurred in World War II with the problem of gas gangrene (clostridial myonecrosis). When definitive surgery following wounding was performed within 24 hours, the frequency of gas gangrene was 0.8%.[10] Among prisoners of war, however, time from wounding to definitive surgery was up to 84 hours. In this time frame, the frequency of gas gangrene increased from 0.8% to 5.2%[10]. A study conducted during the St. Mihiel and Argonne-Meuse operations, September 10 to November 13, 1918, showed that the relationship between the incidence of gas gangrene versus time from wounding is virtually a straight line.[11] The incidence is almost 0% when care of the individual occurs within the first 6 hours, and increases to 75% if the elapsed time is 5 days. This corresponds to an increase of approximately 15% per day of elapsed time prior to treatment in the occurrence of clostridial myonecrosis. No antimicrobial agents were applied in this study. We and others have observed in experimental animals that if you use an appropriate antimicrobial agent in gas gangrene, you can shift this curve and delay the effect by 24-36 hours.[8] The conclusion in this case is that antimicrobial agents do not prevent infection, but they may delay the occurrence and thus provide an extra 24-36 hours to undertake debridement, which still remains the critical factor in terms of prevention of infection.

Several other factors contribute to the predisposition of casualties to infection. These are the number, the location, and the extent of wounds. The effect of multiple wounds is obvious. The effect of location can be illustrated by an example from World War II. Wounds to the buttock area accounted for only 5% of total wounds, yet accounted for over 50% of the cases of gas gangrene.[12] This is not surprising, considering the gross anatomy and the blood supply to the buttocks. There is a relatively limited blood supply through the obturator foramen. If it is injured, a large muscle mass is

devitalized. Associated injuries such as fractures, vascular injuries, and burns also account for an increased occurrence of gas gangrene. An additional study on more recent data from the Yom Kippur war also emphasizes the location of the wound as a predisposing factor.[13] A total of 420 casualties were evaluated, with an overall frequency of infection of 22%. Patients with burns of less than 25% surface area had an infection rate of 14%. However, all patients with over 25%-surface-area burns became infected. Penetrating wounds of the abdomen with perforation of the colon resulted in infection. Surprisingly, fractures resulted in frequency of wound infection of 18% (40% if it involved the femur). These infection rates occurred even in the absence of compound fractures, with the infection rates for soft tissue being lower than for fractures, burns, and penetrating abdominal wounds. Other factors predisposing to infection are general condition of the patient, shock, renal failure, and combined injury (radiation plus trauma). With reference to combined injury, the rabbit model used for producing gas gangrene has provided interesting results.[8] An appropriate antibiotic (one to which the contaminating organisms were susceptible in vitro) could delay, but not prevent, the occurrence of gas gangrene in rabbits irradiated with 650 rads of whole-body exposure. The onset of infection was delayed 36-48 hours, but was not prevented. During these postradiation times, rabbits were profoundly neutropenic. The point is that we could demonstrate an effectiveness of treatment comparable to that seen in the nonirradiated animal during the early time frame following combined injury.

The type, number, and virulence of the bacteria are important variables in the infectious process. Studies from World War II and Vietnam demonstrate that over the time period of the infectious process in the wound, the bacteriology of the wound actually changes.[14] One of the very important observations that has come forth in the last 20 years, and first observed in the Vietnam War, was that the important organisms in causing wound infections were no longer staphylococci or group A streptococci, but were in fact Gram-negative organisms, especially coliforms and pseudomonads.[15] The microbiology of wounds seen in Vietnam that caused problems were Pseudomonas, Enterobacteriaceae, and Klebsiella. This is the group of organisms now seen in civilian intensive-care units, critical care, and burn units. The Yom Kippur

War provided more data on bacteriology. In a total of 420 patients, 178 were infected.[18] Pseudomonas makes up 25%, Klebsiella 15%, Escherichia coli 13%, and other enterobacteriaceae another 7%. This means that almost 60% of the organisms were aerobic Gram-negative bacilli. These organisms are resistant to penicillin, ampicillin, streptomycin, and any other antimicrobial agents that were administered. The origin of these infections is again brought into question. Were these hospital-acquired infections even though it was projected that 9% of them were present on admission?

SOURCE OF OPPORTUNISTIC PATHOGENS IN WOUND INFECTIONS

We became interested in infection in tornado casualties at the time of the 1953 Wooster tornado. In the aftermath of this tornado, the majority of trauma patients became infected. The reason was most likely related to the fact that all the wounds were closed primarily and were not adequately debrided.[16] In April 1970 a tornado struck Lubbock, Texas.[17] In the aftermath of this tornado, more than 500 people were injured and more than 300 treated. Hospitalized individuals had, on the average, 2.6 species of aerobic Gram-negative organisms per wound. Similar observations were made in Vietnam and Israel. Outpatients, on the other hand, demonstrated no Gram-negative bacilli. That suggests very strongly that these were hospital-associated infections.

All hospitalized patients were treated with antibiotics. Most of them received penicillin or ampicillin, in exactly the same combination the British used. No outpatients had received antimicrobial prophylaxis. In hospitalized patients, small numbers of penicillin-resistant staphylococci were found. One would expect this because the patients were treated with penicillin and the resistant organisms were thereby selected. On the other hand, the outpatients had penicillin-sensitive staphylococci. This suggests that the staphylococci grew more rapidly and actually suppressed the Gram-negative flora, with resultant staphylococcal infection in the outpatients and Gram-negative bacillary infection in the inpatients. However, that doesn't explain where the organisms came from. When soil samples from the particular area in which injuries occurred were examined, they were found to contain

Enterobacteriaceae, Pseudomonas, and E. coli.[17] These were the same organisms we found in the wounds. Our hypothesis is that the wounds were contaminated with soil that contained Gram-negative organisms. Treatment with penicillin suppressed the staphylococci. The Gram-negative organisms, which are resistant to the penicillins and some of the most recent third-generation cephalosporins, were selected and were able to grow. The occurrence of Gram-negative infections in Vietnam and in the Yom Kippur War really represents the selection of a Gram-negative flora that was present in the soil. The Swedish Surgeon General has a team studying wounds in Lebanon, where they send individuals out to every site where a person gets wounded. If this hypothesis is true, we may be able to predict the etiology of wound infections before we ever go into an area.

SUMMARY

You can prevent tetanus with a tetanus immunization. Perhaps in the future, monoclonal antibodies of the J5 mutant of E. coli may prevent psuedomonas infections.[18] Limited data suggest that cleaning wounds with Betadine may be more effective than saline alone.[19] Systemic antimicrobial agents will prevent group-A streptococcal infections. How do you prevent wound infection? Debridement! In dealing with the problems of casualties, think in terms of debridement. Make certain that personnel are immunized. Antimicrobial agents may have a role topically, and from limited data they appear to be effective systemically in the irradiated animal for a very brief period of time.

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PANEL DISCUSSIONS

PANEL DISCUSSION

DR. CONKLIN: I would like to thank all of the Panel members and the audience for their participation. I know how extraordinarily difficult it is to get clinicians, especially surgeons, to participate in these forums because of their heavy clinical burdens. So I am very grateful.

The goal of this symposium was to review the pathophysiologic derangements that occur in combined injury. The operational definition for combined injury in this conference is the superimposition of a traumatic, thermal, or radiation insult. One of the reasons for doing that is that we concluded several years ago that there are no useful human data on combined injuries that can help us manage patients. In trying to understand what happens in the very limited animal data, we found very few clues.

INTERDISCIPLINARY APPROACH TO COMBAT INJURY

DR. CONKLIN: One of the underlying foundations in the development of the combined injury program is that the physiologic derangements that occur in polytrauma patients and in bone marrow transplantation patients are similar to combined injury insults. I would like to ask Dr. Santos if that presumption is a useful one or not in trying to develop concepts to address combined injuries.

DR. SANTOS: I think it is. I probably am going to take away an awful lot from this conference because I've learned to think a little broader about the problems we have.

For example, in our patients who are insulted with cytotoxic drugs and then whole-body irradiation, infectious disease is a big problem. So colonization studies would be important. In addition, I didn't look at burns or think about burns, but now I understand that there is a capillary leak syndrome. Well, you should know that we also have one. We know exactly when it occurs. We used to just sort of euphemistically call it a vascular burn, but we don't know that much about it. Wouldn't it be fascinating if it is similar to what you saw in the burn patients?

So, if you take the experience in a trauma center alone, without burns, or you take the burn center and the kind of thing we are doing and begin to look at and test some of these things clinically that have been looked at in animals, I think eventually we will learn a lot more.

DR. WILLIAMS: I agree. I think it is a very, very important concept to look at them in these contexts for their overlap.

As an illustration, I came prepared to believe that the surgeons had not categorized injury. We know nothing about really defining the degree of injury so that we can compare comparable things. I was prepared to believe, for example, that the radiotherapist had this so well controlled that there was no issue, and I have learned that neither of those assumptions is correct. That is of some value, I think, as we look at the rest of the problems. So I think the concept is extraordinarily useful.

DR. McCABE: I can only echo what the previous two panelists have said, that this is a reasonable approach to take. I think the proceedings of the conference will show an exchange of people who represent very diverse disciplines, who have discovered that they have a lot of interests in common and that they are going to become increasingly more common.

DR. LLEWELLYN: I can't disagree with any of the things that the previous panelists have mentioned. I was, however, struck by the diversity of the study interests of the people presenting and attending. I think if you run something like this again, there needs to be a better definition for the uninformed, what combined injury really consists of. I am certain that there are a number of people who are interested in problems of sepsis, derangement of the immune system, and mediators of a variety of kinds, who would not respond to a call for papers or participation in something under this particular heading. For example, I was fascinated by a number of the presentations about mediators from mast cells. In the 2½ years that I ran the Institute for Chemical Defense Research, many of those same kinds of peptide mediators and transmitters were the things we were concerned about from the standpoint of nerve agents.

DR. NINNEMANN: I think if one thing has been made abundantly clear to me during this conference, it is that we are dealing with complex problems, and that is complex with a capital "C."

The solutions aren't going to be easy and they are not going to be the product of a single mind. It is for these reasons that I am a firm believer in the team approach. You need both a clinical side and a basic science side to address problems as we have discussed here.

I think differences of opinion are healthy; they stimulate discussion and may ultimately lead to a solution. We need to realize that pieces of the puzzle that we need to solve our problems may be provided by others such as you who are attending this meeting.

DR. HIRSCH: Perhaps I have a little different perspective. Less than 3 years ago, I came to this organization to ask questions on how to deal with some of these problems, and what was supposed to be a half-hour discussion ended up in 1½ days of discussion. To me it is remarkable that we have gone this far in this very short period of time.

I think that AFRRI serves as a catalyst for this multidisciplinary approach. I hope that AFRRI will continue to be the axis in which all of the different disciplines that usually don't even know each other, much less speak to each other, come together. I think this symposium has done this, and the next one will have to address whatever issues are left undone from this time.

DR. BURKE: I can't add anything but echo the idea that a combined approach is essential. Each of us has to put our own experience in context with all other experiences to interpret them correctly, and this seems to be the proper format to do that.

DR. CONKLIN: Thank you. I would just like to say that I read all of the critiques that were handed in, and everybody has a particular subject of interest. We very intentionally left out chemical agents. We have had numerous discussions with the Medical Institute at Chemical Defense, and we now

have five collaborative studies with them. We are very well aware of degranulation of mast cells post chemical exposure. It will be a major area of collaborative interest. However, I think people have seen just how awesomely complicated the problem is if we only add radiation and a thermal insult together. When we have to add chemical insults to it, it is almost impossible to make order out of what we know now.

The same thing goes for the use of analgesics and anesthetics. We are studying those in conjunction with the Uniformed Services University of Health Sciences here and at AFRRI. We hope to give, in future symposia, some rigorous guidelines about drug metabolism postirradiation and postchemical exposure.

With regard to triage, I spent a great deal of time with a number of the people who teach in our Medical Effects of Nuclear Weapons Course, reviewing what has been done in the last 40 years.

Three years ago I thought perhaps the lymphocyte, which decreases very rapidly postirradiation, might be useful as a biologic dosimeter. Dr. Ninnemann's data, Dr. Munster at Hopkins, and the experiences of Dr. Hirsch in Boston show very clearly that thermal injuries and polytrauma decrease lymphocytes. So one must view the lymphocyte as a biologic dosimeter with some skepticism.

So for triage we can't add anything to it or even make up a matrix. I have done it using prodromal symptoms, nausea, vomiting, diarrhea, etc., but this is probably not useful when you have superimposed another insult on it.

I think the guidelines that are in the NATO doctrine right now are appropriate and should be followed, because there is no current way to assess radiation injury in any of these victims.

IMPORTANCE OF RADIATION DOSIMETRY

DR. CONKLIN: I ask Dr. Santos, who regularly treats patients who have been lethally irradiated, if he would undertake the treatment of somebody for irradiation injury without knowledge of it?

DR. SANTOS: It depends, in a civilian situation; unless I am absolutely sure it was twice the lethal dose, I would do it.

If I were out in the field, I would favor a badge to everybody, but the cheapest kind you can get. I would have it either red or green. If you estimate they got more than 200 rads, I would probably have it red; otherwise it would be green. If you don't make it that simple, then you are going to be doing a lot of pointless surgery. A limited number of personnel just can't spread far enough.

I don't know if you like my first answer--but, quite honestly, if somebody from Three Mile Island called me, we would do the damndest we could. We would make misjudgments. I am not unaware of the publicity one gets from that, and it is important.

DR. CONKLIN: I would like to ask Dr. Mosebar or Major Myers if propositions have been put to the Surgeon's staff or the Army's staff with regard to having dosimeters on people?

DR. MOSEBAR: I proposed on several occasions that everybody be given a dosimeter. At present there is one dosimeter for every 15 people.

We also get into the problem of who would read these, too. My personal feeling in the Army is that they ought to be self-reading.

One entity that hasn't been discussed here today, that we have to take a hard look at on the nuclear battlefield, is the psychological problems. The Israelis had one psychological casualty for every four and one-half wounded in Lebanon. Psychological casualties may well outnumber actual casualties. Who is having radiation-induced vomiting and who is having psychogenic vomiting?

I must say I haven't gotten too far with TRADOC (USA Training and Doctrine Command). We need to look at that group that we can send back to work. I would just as soon have red, green, and yellow designations.

DR. SANTOS: I agree with that.

DR. MOSEBAR: We need a group that can go back to work. I realize numbers are going to be inaccurate on this dosimeter, but at least they are going to give us something to start with.

DR. SANTOS: They are simple to read if they are three-colored.

DR. MOSEBAR: We also need another designation for a medically undecided group. Obviously the Army Medical Service isn't going to take medical steps if we don't have time. Such will be the case with central nervous system radiation patients.

Until now we in the Army Medical Service have said, "Let us have all of your problems." Now we are taking a look at the NBC battlefield and saying to command, "You are going to have to take care of your problems, and we are going to serve as your consultants on the periphery. You are going to have to collect the people. You are going to have to do a large part of the triaging."

Without some kind of a guideline, we are going to be in deep, deep trouble. Our forestructure of the Medical Service is very, very tight, and every time we want another medical person, somebody has to give up an infantry man. The infantry isn't very keen to give up slots to the medics.

We now have 11 percent of the battlefield in the Army Medical Department, and they say that is all we are going to get.

DR. SANTOS: Excuse me. I would like to ask a question.

Obviously I am not into that kind of politics, but one argument is that if you can identify the yellow, it may be cost effective because they can go back and fight.

If it were as simple as you suggested, then you might argue with them if you had the right numbers, you would be able to return X number of troops to

duty. Granted, if you give us someone in medicine, you may have to do without infantrymen. But I think that what you suggested could be argued to be cost effective.

DR. MOSEBAR: There is a study group right now in Washington looking at the chemical battlefield and the question of whether we have enough medical forestructure. We may get a few more positions out of the study. I hope so. The current Chief of Staff of the Army has said that he is doubtful about our medical structure on the future battlefields.

DR. CONKLIN: Dr. Moll (Office of USAF Surgeon General), is the Air Force making any moves to implement personnel dosimetry?

DR. MOLL: No, we haven't. Perhaps one of the reasons is that the opinion has been that it wouldn't make any difference.

NEED FOR STANDARDIZED APPROACHES TO TREATMENT

DR. MOLL: The Air Force has only recently taken combat medicine, or medical readiness, seriously. By that I mean that we are spending about $1 billion between 1980 and 1990. Most of that is being spent in acquisition of beds, medical material, and additional people. Very little of that is being spent in stockpiling medications or specific material for nuclear casualties. The primary reason is that none is available, as far as I know, nor has it been made available to us.

We are stockpiling chemical and biologic warfare antidotes. I was extremely interested in this conference because there were hints of possibly useful nuclear antidotes that can increase your radiation tolerance.

To be more specific, some of the things that especially intrigued me were such things as automatic debridement or a vital tissue indicator. I think the enzymatic debridement or something similar would be much more practical than having surgeons do it.

Bone marrow replacement in the form of safely stored stem cells could help the prognosis if supplemented after radiation. Preventing sepsis, with a specific measure such as Flagyl for abdominal or oropharyngeal wounds, is already being practiced. It is not FDA-approved in this country, but the British used it in their Falkland Islands experience.

The British also stressed the immune system by bleeding everybody 3 days before they went into action. This was possible because they had all of them on the boats when they drew blood. I guess this was a mild stimulus.

Perhaps after bone marrow cells we could look for primordial GI tract stem cells of some sort.

Getting back to automatic debridement, we are going to need equipment that untrained personnel can use because we won't have enough trained surgeons or other physicians to do the job. I would like to have some increased emphasis on the first aid aspects, which include debridement, artificial skin, and the like.

DR. KAPLAN: Enzyme debridement still needs the judgment of someone putting it on. You can put it on, but you need to know when it has done its job and when you have to take over. I don't think that is so immediate a problem in the field type of action. Artificial skin, be it synthetic, semi-synthetic, biological, or whatever, is not a first aid. It is a long-term approach to be used as a patient care facility treatment.

I think one area to look at is what can be done to initially delay some of the techniques that are used. For instance, adding serum nitrate to silver sulfadiazine is used by some people. One of the advantages or disadvantages, depending on your viewpoint, is that it makes an eschar that just sticks around forever. You have to surgically remove it. But perhaps that delay would be beneficial in a military situations, as opposed to nonmilitary.

Looking at what can be done in the field to initially treat those wounds and stabilize them so that you can evacuate them to a field hospital or, preferably, to the continental U.S., is a better long-term solution than working on what can be done right on the field.

DR. BURKE: It's my thought that you ought to be able to develop an enzyme system and artificial skin that could be used on the field.

DR. WILLIAMS: I think I have to also disagree. The thing I have been most impressed with in these 2½ days is that the latent period, the free period, the golden hour, whatever, that traditionally has been of days' duration has been shown to be of, at best, hours' duration.

I think if we are going to make manipulations, the thought that we can stick something on until 5 days later when they go somewhere else is inordinantly naive, given what we have listened to in the last 2 or 3 days.

Thus, I guess I am saying the same thing I think Dr. Burke just said. I really do think we have got to look at doing it very, very, early, whatever "it" is.

DR. VAN DER WAAIJ: I would like to throw out an idea, talking about bone marrow transplantation and self treatment. Shouldn't we consider the possibility of self bone marrow transplantation? You may be aware of the literature saying that if you shield the lymph nodes of a mouse and irradiate the animal at 900 rads, protection is obtained. Should we do some studies on designing a sort of apron shield that shields off the iliac bones and the liver in order to enhance or increase the tolerance for irradiation?

DR. CONKLIN: That is a topic for another forum. Point in fact, we are very, very involved, as is the United States Army, in development of radioprotectors of a chemical nature. In addition, we have been studying lead fabrics and a lithium boride polyester material developed by the Japanese, which absorbs thermal neutrons. We have been characterizing the radioprotective effect of those fabrics with the fast burst reactor at Edgewood, MD, and the TRIGA reactor at AFRRI.

SELECTIVE DECONTAMINATION

DR. CONKLIN: Would Panel members recommend selective decontamination for an irradiated patient?

DR. SANTOS: I think Dr. van der Waaij's ideas and what he presented are very useful, and there are some clinical data for it. Actually this is not how we manage our patients. We wait for the first fever, because we know they are going to be infected.

Concerning the practical problems, I think there is something to be said for selective decontamination. Anybody who has been in the laboratory and has tried to irradiate animals and get them through it to do an experiment has found out that there are a lot of things you did for them. Most importantly, you gave them antibiotics ahead of time to get them through.

A decision based on dosimetry so you wouldn't be passing antibiotics out like candy, might make a difference. I am sorry that there seems to be some resistance to this. But you have to make it simple; red, green, and yellow are very simple. Unfortunately I don't sit in the place that makes those decisions to understand why they don't want them. At any rate, if you can make the decision that soldiers have had a radiation exposure above a critical level, I would be in favor of selective decontamination. That is, I would give them nonabsorbable antibiotics, if possible.

DR. McCABE: I think the approach that Dr. van der Waaij has outlined has really been shown to be extremely effective in patients undergoing cancer chemotherapy. It certainly appears to be rational for people in whom one anticipates granulocytopenia.

I think that it is very important that certain aspects be recognized. One does not try and sterilize the gastrointestinal tract, as was pointed out; one removes only potentially invasive aerobic organisms. Equally important is that it is a nonabsorbable form of therapy.

After years and years of questions about prophylaxis and antibiotic use, it has become clear as to when prophylaxis is effective. Its value is for a limited period only, and it should be very clearly delineated what one is doing it for. It is specifically for the control of aerobic organisms in the gastrointestinal tract; it is not for the prophylaxis of all infections in general.

DR. LLEWELLYN: Selective decontamination seems like a very reasonable thing to do. There are a number of studies that have been done over the last 15 years in other places that have all suggested this sort of thing in a military combat-medicine environment.

You have to think about very simple measures and standardizing them. These kinds of things then have to be put in the hands of people who have go/no-go decision-making capability, people who are really trained as aidmen/emergency medical technicians, etc. The reason they have to initiate these kinds of things is that it may be many hours from the time of the initial insult to the first time that a surgeon is going to intervene, or the first area at which ancillary studies can be done that would guide any kind of further therapy.

To indicate some of the difficulties in even considering this sort of therapy as being within the realm of what the medic could administer, we are just getting to the point now where our medics will actually have intravenous fluids, which they will carry with them and be trained to administer. It seems utterly ridiculous but, in fact, that is how slow the process is for getting these sorts of things put into the system.

Ultimately, things of equal importance to think about are the ways one can exploit the research that is being done on compromised immunity at the basic science level. Perhaps something can be done at the time of the initial intravenous therapy to support the immune system in one direction or the other.

It may be 8 hours before the individual has the first opportunity to get surgery. In the military field situation we have to approach these things in different ways. At the present time it seems that selective decontamination is a very wise thing to do.

DR. McCABE: I think the point was made. I think this is one of the situations in which one is really not talking about emergency care. Selective decontamination is going to be used at the time that the person has received enough radiation that he is going to be granulocytopenic. That is not going to be at the initial period, but several days to weeks down the line.

DR. LLEWELLYN: My response to that is that there are additional reasons for considering selective decontamination that have nothing to do with radiation exposure. If, in fact, you are going to use it, either you have to make it a standard procedure for the medic in the prehospital environment, or you have to restrict it to somewhere further back in the hospital environment.

We have to look at multiple reasons for putting some of these things into the inventory. As pointed out by Dr. Moll, the British did use Flagyl automatically in any sort of abdominal wound. It was part of their protocol to make everything very simple. They had no evidence of any kind of problems from having done this. As a matter of fact, it may very well have been beneficial.

DR. HIRSCH: If the question is "What do we do tomorrow in the presence of five, or six, or even seven patients who may have this problem?" I would pick up the list of participants of this symposium and call up the ones who know the best and say, "Come up and help me out because I certainly haven't got any experience of my own and I would probably get myself in more trouble."

I think the advantage of getting this group together is that in the event of nuclear casualties, resources can be made to study these issues and decide what to do and then make a decision as to what to do for standardized

DR. BURKE: The concept is certainly a correct one, but I don't know of any mechanisms that we can use clinically, effectively now. So I wouldn't do it now, but I think this is an area that ought to be looked into extensively to see if there isn't some way to get at it.

MEDIATOR MODULATION AND IMMUNOTHERAPY

DR. CONKLIN: Dr. Burke, could you comment on some of the things that Dr. Fletcher reviewed this morning, especially the nonsteroidal anti-inflammatory agents?

DR. BURKE: The concept is a very interesting one and perhaps a very important one. At this minute there isn't any clinical mechanism that I know about that would allow me to use it. Perhaps in the future--now, no.

DR. CONKLIN: Dr. Hirsch?

DR. HIRSCH: The same.

DR. CONKLIN: Dr. Ninnemann?

DR. NINNEMANN: I kind of lump the two together for simplicity, mediator modulation and immunotherapy. Looking at it as a basic science immunologist, I am going to talk around it for a minute here.

In an evolutionary sense, the immune system evolved to help man cope with microorganisms in the environment. We have to remember that we are in delicate balance with our environment and with the microorganisms in that environment.

Nature didn't foresee certain things like, for instance, transplantation. The allograft response had to evolve for another purpose. It isn't there to resist transplants. Nature didn't foresee that. I think, in another sense, nature didn't foresee major combined traumas, particularly major combined traumas involving radiation.

What we have, in fact, is a twofold problem in trauma, mixed trauma, for which nature didn't provide a solution. First of all, there is a surgical problem. We have anatomical repairs; we have physiologic systems that are damaged that have to be repaired and have to be repaired quickly. That has been pointed out very clearly.

Second of all, there is a basic science problem. The balance between nature and microorganisms has been altered. That is a problem. At this point we are dealing with immunologic, biochemical system, and mediator changes.

I can't address the surgical, but I can address the basic science problems. From my point of view there are three purposes in studying combined injury and immunologic changes that go along with it.

First of all, we have to define the defect. We already know the consequences of an immunologic defect in patients with traumatic injuries. But we haven't, in many cases, adequately defined the defect itself.

Second of all, we have to delineate the cause of the defect. We have to know the mechanisms. We have to know why the defect is there.

The third thing that we need to do is to devise methods of therapeutically correcting the defect. Only when the first two conditions have been met can we get to the third, where we can start therapeutically manipulating the immune system.

There are some exceptions to this. I think Dr. Braude's presentation pointed this out, of altering the balance in favor of the host. But I think we are not ready for modulation of mediators, or immunotherapy. The basic understandings are not complete enough to start playing with the immunologic system and manipulating systems within the host.

A good example of what happens when you try to do that without the basic knowledge is what has happened in the Cancer Program. Ten years ago immunology was supposed to cure all of the cancer problems. There was a great

deal of optimism in the Cancer Program. Immunology has fallen into disrepute because it has not solved the problem. The expectations were too high; the basic knowledge was not there.

I think we are on the threshold of new possibilities. There are things available now that haven't been available before. There is a great deal of advanced technology that can be applied, and we are getting a very much better understanding of the immune system. While I don't think we are ready to start diving in and manipulating yet, I think that will come about in the near future.

DR. LLEWELLYN: I share your sentiments very, very strongly. Since most of my experience has to do with research against chemical warfare agents, nerve agents in particular, I want to draw a parallel. When I took over the Medical Institute for Chemical Defense we were supposed to work on antidotes against these agents. I was presented with what was essentially common knowledge; everybody knew how the agents worked. They were anticholinesterase agents. People were certain that they could design various kinds of drugs that would interfere with these and peel them off. It made an epidemiologist like me think that they viewed the body as a uniform test tube and that there were very simplistic, very direct sorts of things going on, as opposed to this cascade of innumerable mediators, modulators, transmitters, suppressors, enhancers, etc.

I think if you pay close attention to the neurobiological literature over the next 3 years, you are going to see most of our understanding of the cholinergic system completely rewritten because people have gone back to question a lot of what we thought we knew.

Now, when you are talking about something like nerve agents or an organophosphate, a simpleminded person like me would first say to a pharmacologist, "Now, where does this go?" The answer would likely be "Well, it has to go to the endplate, the juncture." Yes, it probably does. It also goes to all kinds of other places. As a matter of fact, nobody knows all of the places it goes.

They don't know what form it is in when it goes to those places. So all of these mechanistic things that you are talking about are just terribly critical. Otherwise, what you have is some kind of symptomatic intervention. It may be all well and good to, in fact, treat symptoms in a number of cases. But I would share your concern about not launching into something of this type without considerably better understanding of all of the things that are going on simultaneously. I don't mean to unsay the things that I said before about why, from the very practical standpoint, I think we should probably consider selective decontamination, even if you don't expect radiation on the battlefield.

DR. CONKLIN: Dr. McCabe, can I refocus the question a little bit since Dr. Braude is not here? Some of his work was very tantalizing. There are other groups that have worked in this area like yourself. Should we be looking at this area, particularly at passive immunization in combined injury more aggressively than we are now?

DR. McCABE: I think there are two points. I think the mediator modulation is very intriguing, but it is many years too premature to consider it as a practical method at this time. It needs a lot more in vitro study and field trials, etc. In contrast, I think the question of immunotherapy has fairly firm experimental basis, and Dr. Braude has provided us with some very good clinical information that there is considerable potential for passively administered immunotherapy.

Perhaps even more practical might be considerations of active immunization, if we can answer some of the problems that I don't think time permits us to go into. Historically, active immunization has worked a lot better than passive immunization, and I do think there is potential for this.

DR. WILLIAMS: Dr. Burke showed a patient on whom he put his special graft, and told us the man should have been sick, but he didn't know he was sick. We don't know if these patients who are very sick should be made to feel like they are well, and we need to know some of these things.

DR. SANTOS: I will keep with my reputation and give a mixed answer. Most of my academic life has been spent in immunology and cellular engineering, trying to understand what is going on. We think we know in our patients what the defects are immunologically, at least within limits, as far as handling cytomegalovirus infections. If marrow transplant patients are infected, as defined by positive culture from the blood or their marrow, not shedding in the urine, we can now identify a group. If they develop NK cells, or T-cells that are capable of lysing their own fibroblasts infected with CMV, we can put them in a column where 95 percent will survive. This has recently been reported in The New England Journal of Medicine. If they are unable to do this, it means looking into a defect in interleukin-1, or interleukin-2. These are things we can manipulate.

We might be interested in some clinical trials because we have defined what parameters we are looking at. I am not convinced that I have heard anywhere that classical immunology, not taken in a broad sense, has anything to do with bacterial infections. I want to see those data. You know it is motherhood to say it is good. This was the problem in the cancer business. I stayed with that for 2 years, and then I stopped getting into immunotherapy in cancer.

DR. McCABE: What is more classical than the demonstration of antisera to pneumococcal capsule?

DR. SANTOS: I am sorry--antibody aside.

DR. McCABE: Now you are talking about cell-mediated immunity. I agree with that entirely.

I brought this up earlier and I would strongly agree that with classical bacterial infection I haven't seen any good evidence that T-cell mediated immunity has any protective activity.

DR. BROOK: Immunity, using passive immunization or active immunization, is probably going to work in a limited type of situation, that is, accidents or a limited nuclear war. But when you are talking about all-out

war where you have thousands of casualties, and where medical supplies may be cut and medical personnel unavailable, and there are chaos and disorganization, the most practical and efficient way for it to be carried out by soldiers and civilians may be with antimicrobial agents.

Selective decontamination, in my judgment, probably prevents bacteremias or septicemias in individuals who have been immunosuppressed because of irradiation. When dealing with combined injury where, in addition to the leukopenia and total-body irradiation there is also trauma (especially intra-abdominal trauma, trauma around the oral cavity, where you get in contact with normal indigenous flora), you are dealing with a situation in which selective decontamination won't do the job.

You need antimicrobial agents to help the patient survive skin infection with Staphylococcus aureus, intra-abdominal abscesses due to Bacteroides, or infections of the oral cavity. Like soldiers who sometimes carry in their pockets atropine ampules, against gas, they could also carry an ampule of antimicrobial agents. It will be important to have preparations such as a combination of ampicillin-clavulanic acid, which has very wide activity against staph, strep, Pseudomonas and anaerobes.

DR. McCABE: I think that is a very nice thought. When one talks about using antibiotics and trying to dispense it to the general medical population, it is almost impossible to do it with corpsmen passing out pills for everyone who feels ill. I am convinced one will kill more people than they are ever going to save by such an approach.

I think it may be effective in selected instances. There is no question that antibiotics are necessary because people are going to get infections.

WOUND CLOSURE

DR. CONKLIN: I thought some of the concepts concerning closure that Dr. Burke and Dr. Hirsch presented were very provocative. Is this approach valid, does it have utility, and where should we go from here with it? Should

this be an area of aggressive research, or is it totally unacceptable and repugnant to military battle surgery to even consider these kinds of therapeutic interventions?

DR. HIRSCH: Were I to be faced, today or tomorrow, with one or two or three patients who were involved in some sort of military or civilian accident, involving some amount of radiation, somebody would have to tell us if it was a lethal dose. If a nonlethal dose, I would do as much surgery as is humanly possible to correct as many defects as I possibly could. I would not be concerned with trying to salvage areas of the body that are not needed for life. For example, a bad extremity should be converted to an amputation with a clean surgical wound that can be closed instead of going through a number of open wounds that require dressing. More aggressive life-over-limb treatments are needed in these situations. I would support the patient with intravenous antibiotics to provide the best "sterile environment" possible. By doing this we have been able to reproduce some of the eastern European experience, or German experience, and salvage some of the patients.

Then somewhere, around 4-6 weeks, the likes of Dr. Santos will have to deal with the hematological possibly infected patient.

DR. SANTOS: It is too late then. Don't call me then.

DR. HIRSCH: I will call you earlier, but I am saying surgically, we hope the problem is over.

DR. BURKE: I think that my point of views are fairly straightforward about this. Rapidly getting rid of necrotic tissue, infected or not, and closing the wound have got to be the first moves.

Surgical interventions, including antibiotics at the time of injury, are here to stay. I don't believe that those are going to go away or be replaced by any amount of other kind of therapy.

DR. WILLIAMS: Everybody has agreed that we need perfect resuscitation, perfect debridement of necrotic material, and then physiologic restoration, but we don't know how to do that. I think that there are a few things that we have learned.

For example, if a person has a ruptured spleen, should we take a long time to try to conserve that spleen, spending a lot of resources and risking a second hypotensive episode? Or is that so unreal that we should just do the splenectomy? I don't know of we know that kind of thing, and we need to. I am not aware that we have data as to how securely a properly done colon anastomosis heals in an irradiated circumstance. We need to know that.

Skin coverage has been mentioned many times. The traditional method of skin coverage before we had artificial skin was to take a skin graft. But taking a skin graft induces a wound in its own right. That wound may be as much trouble as the one you covered. If that is true, then the whole issue of artificial physiologic wound coverage becomes ever more critical.

There were at least two presentations that showed that very early surgery, occasionally before the irradiation or afterwards, actually improved survival. I suspect it means that necessary surgery done perfectly ought to be done early. That is probably pretty secure. What you do, then, is not nearly so secure, as I see it.

DR. LEICESTER: As we have been quoted from our work in the Falklands, I would like to bring up a couple of points and also say how much I enjoyed Dr. Burke's and Dr. Hirsch's ideas about early skin cover.

Although we have had no experience in combined injury in terms of irradiation, we did have 110 patients with burns whom we treated on the hospital ship. Approximately 40 percent had combined injury in terms of blast, shrapnel, or thermal injury.

Our policy, as Colonel Llewellyn pointed out, was that everybody received prophylactic penicillin and tetanus toxoid. How this interferes with immunotherapy, I don't know. Whether it was necessary, I don't know, but we

worked on a simple policy. We had a simple resuscitative policy of a liter of Hartman's solution followed by colloid if it was needed.

We didn't have any problems with infection. Large burns in combination with other wounds were treated as a dirty contaminated wound, and we practiced early excision. We didn't have the artificial skin cover that Dr. Burke has been developing, but we covered it with silver sulfadiazine and dressing, which effectively closed the wound. We did remove all of the debris first.

I think that our figure of mortality was 0.4 percent in 730 patients. I think that these techniques of early surgery and antimicrobial prophylaxis certainly contributed to this.

DR. KAPLAN: Was the surgery done by surgeons or corpsmen?

DR. LEICESTER: The surgery was done by surgeons, including a plastic surgeon who had a lot to do with the burns. I might add, that we did a lot of early skin grafting using mesh grafts. This actually is not difficult. We taught all of the junior surgeons to take skin grafts, and once you have meshed it, it covers a big area.

DR. HIRSCH: The hospital ship on which you were working--was it the Uganda?

DR. LEICESTER: That is right.

DR. HIRSCH: That hospital ship, for all practical purposes, was activated as a hospital ship for that occasion. So for all practical purposes, there were no nosocomial infections present on that ship. All of the medical activity began with the military engagement and finished shortly thereafter.

DR. LEICESTER: Yes, that is right.

DR. HIRSCH: The experience that Dr. Walker and Dr. Myron Tong had in Vietnam in 1969 was that if you take severely injured people, resuscitate them, clean and wash them, and then culture their wounds, there are very few, if any, bacteria present. As the days go on in the hospital, the whole bacterial zoo develops. Dr. McCabe, who probably has the most nosocomial infection experience in the world, keeps telling us that the worst place to be is the Intensive Care Unit of a hospital.

Perhaps the immunosuppressed patient should not be treated in a hospital. It is probably better to be operated on in a kitchen of somebody's house, because being in a hospital exposes you to a number of undesirable things. All of us recognize that sepsis in the Intensive Care Unit is much more prevalent because of its invasive nature and the concentration of people.

The type of environment should be in the list of things to address for the next program. The hospital Dr. Leicester is talking about was created for this event and didn't last very long, 2 weeks.

DR. LEICESTER: I don't know how many of you on this side of the Atlantic have seen the conditions at Ajax Bay. It was a filthy, disgusting environment full of dirt and dust; doors were kept open and the wind blew through, and yet the infection out of that was virtually zero.

DR. CONKLIN: There was some material presented with the use of clavulanic acid as a way to decrease beta lactamase and things. In that kind of environment, would any of the Panel members use that kind of agent in conjunction with their antimicrobial regimens?

DR. CONKLIN: A unanimous no.

MODELS FOR STUDY OF COMBINED INJURY

DR. CONKLIN: Another major goal that we have is the model for systems that were being studied. We chose the canine with our radiation studies and combined insults, for a number of reasons.

It has an LD50/30, not too dissimilar from man. It is a large mammal. We wanted a dose of irradiation that depressed the hematopoietic system, reducing stem cells to the neighborhood of 10 percent, but not with an excessive lethality. The dose of 150 rads was chosen because of the NATO/STANAG agreement 2083. Radiation Emergency Status Group 3 is the dose at which the commander doesn't commit his troops to battle except in extraordinary emergencies.

In subsequent studies, particularly burns, blunt trauma, and ballistics what animal model would you select?

DR. SANTOS: The canine appears to be a good model, although some people are using the rat. There are principles that one can gather from the rat. I wouldn't go to monkeys and so forth just because they look like man. I would also use man.

We don't like combined injury with a marrow transplant patient, but it is rare that we have the combined injury you have defined here. However, that source can be used for trying to understand radiation, dosimetry, etc.

Dermatologists argue for the pig. There are advantages for using the pig as an alternative to the dog. A rodent like a rat can be used in a model system. It costs more than a mouse, but it is a little easier because of its size.

I don't see any reason for using monkeys. They bite, they are hard to keep, and they are costly.

DR. CONKLIN: Dr. Valeri, do you want to address why you use baboons?

DR. VALERI: We don't irradiate baboons.

DR. CONKLIN: I am not picking on Dr. Valeri, but a question has been raised that we ought to be studying hemorrhagic shock in conjunction with radiation in subhuman primates.

DR. VALERI: All of our experience over the years has shown that the baboon is very similar to man. Many of the experiments that we do, unassociated with irradiation, are very predictive as to what happens in man.

DR. SANTOS: So does the rat.

DR. VALERI: I am talking about the experiments that we do in baboons with regard to blood products and hypothermia.

The information we obtain is very applicable to man; and therefore, over the past 5 or 6 years, we have replaced the baboon with normal volunteers for the evaluation of blood products.

Talking specifically about irradiation injury in an animal model, clearly, the canine is ideal because you can give him total-body irradiation, which we have done, and you can reconstitute him with peripheral blood stem cells. I also support Dr. Santos' idea that we do our current work in man.

We use the baboon primarily because the baboon, in our hands, with regards to bone marrow and peripheral blood, is very useful. So we can, under very established conditions, identify conditions to cryopreserve peripheral blood and/or bone marrow stem cells.

With regard to your model development, we have a hard time convincing ourselves that we should be irradiating baboons and subjecting them to thermal injury, hemorrhagic shock, and so forth. It is almost impossible to use that animal because you have to put it to sleep.

DR. WILLIAMS: I think the possibility of the pig has already been mentioned, with regard to both hemodynamics and skin. They are much like men, but I don't know about the other systems. I suppose we have to worry about that.

With respect to models rather than animals, I make two or three points. With an interest in mesenteric ischemia, I worried about that issue. Having seen what is here, I am not certain that some of the models don't have to readdress the issue of gastrointestinal ischemia as an important component.

I think in some of the cases where we are looking at two injuries, not necessarily done at exactly the same time, we have to worry about the restressed models, that is, what happens when you add one stress on an animal that has already been stressed from something else.

Two other quick observations: Concerning splenic trauma and its surgical removal (a previously common operation), we should now consider the function of that spleen and its various components. Lastly, I think we have to worry about what happens to bowel resections in the presence of irradiation.

DR. McCABE: I would like to jump from your topics to make a plea for another type of model, a more reasonable model of infections in experimental animals. It is very difficult to find very many model infections that are truly infections in which the characteristic is that for death to occur, small numbers of innoculated bacteria must multiply to large numbers. There is a tremendous tendency to use models in which one infuses 10^{10} bacteria. That is about the size of the federal deficit, as I recall.

For example, for small rodents if you exclude the burn model and one or two species of gram-negative organisms, it is very hard to find one to go along with the other models that you are studying. One needs to look for different organisms and ways to make more realistic infection models.

DR. CONKLIN: One of the major efforts of our program has been the development of a consistent and reproducible sepsis model. Dr. Walker has been working on that for a year and a half in the canine. He presented two approaches; one we found very early didn't work well, with injection into the anti-mesenteric side of the bowel wall. The other was a fibrin clot with the E. coli in the clot.

DR. WALKER: Dr. Fink has really been doing all of that work lately. I just supply bacterial cocktails to him. But you are right; we have been trying to work with those models to see if we can get something that would be more akin to a natural type of infection.

The problem that has been bothering me for years is that we take models and test them with 10^{10} bacteria, and the types of pathogens we are studying are those that don't affect a normal, healthy host. What we need to do is find ways to compromise the host and either put our infection artificially into it or, alternatively, colonize this host in his gastrointestinal tract and let it develop a more natural type of infection. As I said yesterday, that is the way I think infections occur in these types of hosts. We don't have a perfect answer yet, though.

DR. McCABE: I think that does answer it, as we have both said the same thing. We have to try to develop models that are models of infection rather than intoxication, and I think we are working toward those.

DR. LLEWELLYN: I strongly agree. I chaired a NATO conference in Washington in 1980, and it reviewed models that had been used in chemical warfare research.

It turned out that everybody had a very good rationale for whatever kind of rodent, subhuman primate, or other model they used. In fact, they hadn't the foggiest notion of how one model compared with another, and generally they had not reviewed the literature to be able to see that some of the models that were useful for describing the effect of the agent were of utterly no use in evaluating the effect of therapy.

So it depends on what you want to use a model for. In some cases, subhuman primates may, in fact, be the best ones, but you had better have a rationale for it. Having fought that battle on both sides of the bench, both to get my protocol supported and then trying to talk other people out of doing it, I realize it is an extremely difficult sort of thing to do. We need to be

clear about what we want the model for, and then not take a model that is useful in one sphere and use it for something else for which it may be inappropriate because of different metabolism of drug or whatever.

DR. BURKE: It seems to me that the only universal model for the study of humans is, in fact, humans and that is really very awkward because there are all kinds of human study problems that are completely insurmountable. We have got to have an animal model.

What we have tried to do is have one animal model for everything. That clearly does not work. If we are going to study lymphocytes, we had better figure out exactly what we want to do and what questions we are going to ask, and then look for the appropriate model.

The model business is going to continue to be very difficult; the model may change every time your questions change or your problems change. Finally, we ought to work very hard at developing systems to reasonably carry out human studies in an acceptable way, because I think that the most straightforward information is going to come from these studies rather than from developing a model that is going to be only an approximation of the questions asked.

DR. HIRSCH: I totally support that. I think, though, that there are two things to address here. What we have heard from the surgical side is that perhaps there are some new and different technical concepts to be taught to practicing surgeons. I think it behooves the leadership of this group to address not only the issues of result but also, if spleens are to be salvaged in these patients, the need for more education on how to repair spleens. If the issue is closure of wounds within 24 hours of injury, an effort must be made to teach surgeons. So it goes for every other type of injury that one can think of, and that will take time. If it takes 15 years to get corpsmen to start IV's, it will take surgeons some time to learn how to change their provincial thinking.

I think there have to be two avenues; one is to develop a model that addresses some of these questions, and if the results look like they are promising, then an education of these techniques should be started so that the lag phase is not too long.

DR. NINNEMANN: I would like to partially echo what Dr. Burke just said.

An example is the burn work that we have been doing. For certain things, miniature swine works very well. It is a good resuscitation model. The architecture of its skin is very similar to the human's. Itscardiovascular system is very similar, and they have similar eating habits to a lot of humans.

I think it is very clear that T-cell function is very important for the clearance of certain intracellular parasites, particularly viral and fungal infections. And the entire immunoregulatory network is a T-cell network. I think that is important to remember.

If you want to look at T-cell function, the pig is not a good model to study. You cannot raise a mixed-lymphocyte response, and you cannot mitogen-stimulate pig cells.

What I am trying to say is this: know what system you are going after, and make sure the model that you are using to study is appropriate for looking at that system. You are not going to find a model that is exactly like a human. No other animal is exactly like a human. So a model that is appropriate for cardiovascular studies may not be appropriate for immunolgic studies, etc.

DR. KAPLAN: I agree with Dr. Burke that the human is the best model. Occasionally we do have an opportunity to have human models. One of the greatest advances in the true scientific care of the burns occurred in Boston, many years ago, with the Coconut Grove fire. It was very unfortunate, of course, that the disaster occurred, but fortunate that it occurred in an area where people like Dr. Moore, Dr. Cope, and others were and gained the knowledge as well as the care of the patients.

I hope that after a disaster such as the Falkland Islands, they can go back and look at what was done right and also what was done wrong and should be changed next time. I think it is very important, particularly in a disaster-type situation, to take a close look as it occurs, if possible, to gain what benefit we can from those events.

DR. CONKLIN: Thank you. Parenthetically, when we compared our data a year ago with what Drs. Hirsch and McCabe were doing in the Boston University Medical Center trauma program, we were very gratified to find that almost every system that we measured paralleled what they were seeing in their multitrauma patients.

I hope that we will see a similar response in the bone marrow transplant patients. We are looking at similar types of phenomena, and we can learn a great deal from parallel kinds of events.

THYROID FUNCTION

DR. SANTOS: Have the people who studied this massive trauma, or these massive burns, studied thyroid function?

DR. HIRSCH: Ours was normal.

DR. SANTOS: In our irradiated patients, it does go down quite a bit and it is a low-dose rate. We are wondering if that is playing a role in some of our sick patients. What about some of your animal studies here?

DR. CONKLIN: We have not yet examined thyroid function, although there is a proposal to do that.

CATABOLIC ACTIVITY

DR. CONKLIN: Last month in The New England Journal of Medicine, Dr. Cloughs reported some work on a small circulating peptide in some of his burn

patients. Dr. Barachus reported some other observations in the next article with regard to increasing catabolic activity, which goes along the lines that Dr. Dudrick talked about this morning.

The interesting thing about the Barachus article is that some of those events were interdicted with prostaglandin synthetase inhibitors. We have ignored nutrition totally in terms of therapy. Do any of our colleagues here have some comments?

DR. HIRSCH: I was a little mystified by Dr. Dudricks' lack of a more in-depth discussion of the enteral route for nutrition, particularly for the type of patients we are talking about here.

Intravenous hyperalimentation is obviously a very successful modality of therapy, and a lot of people are alive today who would not be alive without it. However, there are a lot of complications with it, and it requires a rather sophisticated staff and support systems so that things can be prepared not only to be administered but also to be followed, so that you don't do bad things. Industry is coming nearly on a monthly basis with another mixture in flavor X or flavor Y that is an improvement on the previous mixture.

I think that the GI tract for these patients and for many other traumatized patients is probably the best possible route for alimentation. In some ways, Dr. Clough proved that in some of the work that he has done. This polypeptide that he has identified is less active in the patients who are stressed by enteral effect than patients who are stressed by intravenous effect.

I think another topic for the next of these meetings is the enteral route of nutrition.

ABSTRACTS OF POSTER PRESENTATIONS

EARLY EFFECTS OF GAMMA RADIATION ON RAT KIDNEY FUNCTIONS

L.M. Amende, D.L. Kelleher and M.A. Donlon. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

The effects of whole-body gamma radiation (900 rads, ^{60}Co) on rat water intake and renal function were examined. Water intake, urine volume and specific gravity, osmolality and urinary protein were monitored during the first 24 hrs postirradiation (PI). Rats were housed individually in metabolic cages. Urine was collected hourly for 3 hours, and at 6, 9, 12, and 24 hrs PI. All data were compared to sham-irradiated animals. Significant increases in water intake were observed at 2 hrs PI, with maximal increases occurring at 3 hrs. Increased water intake continued for 24 hrs. Urine volume increased significantly by 3 hrs PI and remained elevated for 24 hrs. Three hours following irradiation, urine osmolality and specific gravity decreased significantly; maximal changes occurred at 6 hrs PI. Urine osmolality and specific gravity returned to sham-irradiated levels by 24 hrs. Total urinary protein excretion was significantly greater by 2 hrs in irradiated animals. The elevated urinary protein continued for 24 hrs (4.05 $\pm$ 0.144 vs. 2.04 $\pm$ 0.169 mg protein/24 hrs). These data indicate radiation-induced alterations in kidney function occur within the first 3 hrs following irradiation. (L.M.A. is an NRC-AFRRI Fellow).

SWINE SKIN HISTOLOGIC PARAMETERS: A MODEL TO EVALUATE THE EFFECTS OF IRRADIATION IN VIVO AT THE CELL LEVEL

J.C. Archambeau. Director of Radiation Research, City of Hope National Medical Center, Duarte, CA 91010.

The erythema, desquamation and nonhealing produced by irradiation serve effectively to characterize and compare different radiations and time exposure schedules. However, they provide little information about the response of epidermal and endothelial populations producing these changes. Such data are lacking and have to be available if the skin is to remain a useful in vivo test system. This work reviews the histologic parameters of the nonirradiated skin and summarizes the epidermal and endothelial population changes produced by single exposures of 1700, 2300 and 2700 rads.

The epidermal basal cell density is 2031 $\pm$ 48 basal cells/cm with an average cell cycle time of 12.3 $\pm$ 2.4 days, and a growth fraction of 1. Following irradiation there is a nondose dependent linear decrease of basal cells to about 20% of control by 21 to 25 days. This nadir is followed by a dose dependent exponential repopulation to control levels by day 28 to 32. The average cell cycle time is 13.6 hours; three subpopulations are identified with D_0's of 272, 568, and 1620 rads.

The dermal endothelial population density is 2020 cell/cm within a 0.35 mm of the basement membrane, the tritiated thymidine labelling index is 1.5%, and the mitotic index is 0, indicating a prolonged average cycle time. Following irradiation the endothelial cell density remains around control levels until day 28 to 32 when there is an abrupt decrease to 50% of control at 1700 rads and lower at 2300 and 2700 rads. This population decrease is accompanied by a second decrease in the epidermal population. No endothelial repopulation is noted out to 70 days.

The model is suitable for combined studies, however, it is work intensive requiring a large animal facility. Cost benefit ratios have not been determined. A fifty kilogram female Yorkshire swine costs about two hundred dollars with a maintenance charge of five dollars per day. The above work required 23 animals maintained 13 weeks for the irradiation studies, and 53 animals for 4 weeks in the nonirradiated studies, at a total cost of $33,000 in 1982 dollars.

EFFECTS OF BIOLOGIC, BIOSYNTHETIC DRESSINGS ON WOUND TISSUE MACROMOLECULAR SYNTHESIS

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These studies were conducted to compare the effects of biologic (BD), biosynthetic (BSD), and synthetic dressings (SD) on tissue generated in full-thickness skin wounds in rats and guinea pigs. In some experiments, rats received two 4 cm^2 full-thickness skin wounds, one skin removal, the other a 3rd degree burn followed by immediate excision. In other experiments, 4 cm^2 wounds were made in the mid-dorsum of 72 rats and a dressing (viable or non-viable skin; a silastic membrane-covered nylon coated with collagen peptides, D_1; a polyurethane film, D_2; a hydrogel, D_3; or fine mesh gauze, D_4, n=6/group) was sutured in place. Animals were sacrificed on days 3 and 5 postgraft and portions of the wound bed removed for biochemical analyses. In a second series, 15 cm^2 wounds were made in the dorsal skin of 84 guinea pigs and dressings placed. Animals (n=3/group) were sacrificed and samples collected at 1, 2, 3, and 5 weeks postgraft. Results of the burn experiment indicate that viable and nonviable dressings are equally useful as temporary wound dressings. The architecture of the wound tissue beneath BDs and SDs is different; the former is thin and high in collagen content (viable group day 3, $\bar{x} \pm SD$, ug collagen/mg dry weight 143.3 $\pm$ 77.9), whereas the latter is usually thickened, highly cellular, sometimes stratified and richly vascularized, and relatively low in collagen content but high in noncollagen content in the first week after grafting (D_1 38.4 $\pm$ 24.0; D_2 33.37 $\pm$ 15.24; D_3 26.07 $\pm$ 10.93; D_4 37.92 $\pm$ 26.81, for each value compared with the viable group $p<0.01$). A similar trend was noted for collagen synthesis as well. Results of the guinea pig experiments confirmed and extended the original observations. DNA and protein quantity and synthesis were measured for each group for up to 5 weeks after grafting. DNA synthesis for SD groups was equal to or greater than that for the BD group (autograft), particularly beyond 2 weeks. Total collagen content was less in the SD groups than that for the BD groups during the first week, but increased to values equal to or greater than that of autograft by 5 weeks postgraft.

Conclusions. Wound cells in a full-thickness defect react differently to a BD vs. a SD. Tissue architecture is different; more tissue is produced in the wound covered by SDs, but in the first week, less collagen is present. By 3-5 weeks postgraft, the wound tissues beneath SDs are similar biochemically. Therefore, in comparison to the autograft control, the greatest disparity in wound tissue character induced by SDs occurs within 1-2 weeks and may be normalized subsequently.

ADRENAL CORTEX RESPONSE TO COBALT-60 GAMMA IRRADIATION-MORPHOMETRIC EVALUATION

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Guinea pigs received a single, bilateral, whole-body dose of 2.00 Gy. The animals were sacrificed at 2, 3, and 4 days postirradiation and the adrenals prepared for electron microscopy. The ultrastructure of the cortical zona fasciculata cells was evaluated by morphometric analysis. A persistent increase in the mitochondrial area fraction is observed and on day 3 becomes significant ($p < .001$). The area fraction of the vacuoles increases initially (day 2), decreases on day 3, and both organelles return to normal values by day 4 (Fig. 1). The ratio of mitochondrial-vacuolar area fractions reveals a very significant variation on day 3 ($p < .001$) (Fig. 2).

Conclusions.

1. Electron microscopy is an effective tool for the evaluation of ultrastructural response to radiation.

2. There is a very specific tissue-dependent, interaction between the mitochondria and the vacuoles in the adrenal zona fasciculata cells.

3. Mitochondria and the mitochondrial/vacuolar ratio are very sensitive indicators of gamma radiation exposure.

ANATOMICAL GLYPHS FOR PATIENT TRACKING AND AUDIT IN THE INTENSIVE CARE UNIT

H.R. Champion, W.J. Sacco, S. Fallon, S. Morelli.

The researchers have developed detailed patient triage, tracking and evaluation of care rationales which combine the Trauma Score, Global Score and the Injury Severity Score. Use of these tools is illuminated and enhanced by anatomical glyphs, pictorial displays of patient condition. Anatomical glyphs allow data to be easily interpreted and provide a compact characterization of patient course and patient transition as a function of intervention. They are easily computerized. Various combinations of anatomical glyphs are displayed that can assist the intensive care clinician to quickly and accurately view patient condition through assessments of cardiovascular, renal, hepatic, and central nervous systems.

EVALUATION OF TRAUMA CARE

H.R. Champion, W.J. Sacco, S. Morelli, M. Golocovsky.

Evaluation of the effectiveness of trauma centers and trauma systems is a crucial issue in health care system development. This effort has been hampered by the unavailability of methodologies that accurately control for patient severity and case mix, differences in hospital coding and recording systems, etc. Here we present the application of a methodology for the evaluation of emergency trauma care that controls for patient severity, reliability of the indices, and the match between patient samples. The methodology incorporates both anatomic and physiologic measures for a qualitative and quantitative system evaluation. The methodology is demonstrated for intra-hospital trauma evaluation at a Trauma Center and at a Community Hospital. Display of results allow a hospital to quickly identify patients whose outcomes appear to be anomalous. Once identified, these cases can be audited in detail. The methodology is also applicable to pre-hospital and systems emergency care and can be used to identify trauma system strengths and weaknesses.

THE ROLE OF THE TRAUMA SCORE IN THE TRIAGE OF MASS CASUALTIES

H.R. Champion, E.J. Sacco, M. Golocovsky.

Reduction in the time interval between injury and appropriate treatment has been demonstrated consistently to improve survival following major injury. Efficient and effective triaging mechanisms can facilitate the triage process. However, effective triaging in disaster circumstances is extremely difficult, due to the large number of casualties involved, exhaustion of nearby hospital resources, and time/distance factors. Here, the use of Trauma Score for patients with penetrating or blunt injuries is presented. The Trauma Score was applied to a data set of 3,000 penetrating and blunt injured patients. Results show that the Score is a powerful predictor of mortality for both penetrating and blunt injured patients. A triage decision rule, which incorporates the Trauma Score, is presented along with a two-step disaster triage system.

POSTRADIATION INCREASED INTESTINAL BLOOD FLOW BLOCKED BY ANTIHISTAMINES

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Radiation-induced systemic hypotension is accompanied by increased intestinal blood flow (IBF) and an increased hematocrit (HCT) in dogs. Histamine infusion leads to increased IBF and intestinal edema with consequent secretion of fluid into the intestinal lumen. This study was performed to determine whether these effects could be diminished by prior administration of H_1 and H_2 histamine blockers. Dogs were given an IV infusion of mepyramine (0.5 mg/min) and cimetidine (0.25 mg/min) for one hour before and for one hour after radiation (H_1 and H_2 blockers, respectively). Mean systemic arterial blood pressure (MBP), IBF and HCT were monitored for two hours. Systemic plasma histamine levels were determined simultaneously. Data obtained indicated that the H_1 and H_2 blockers, given simultaneously, were successful in blocking the increased IBF and the increased HCT seen after 10 K rads, whole-body, gamma radiation. However, the postradiation hypotension was unaffected, with the MBP falling to a level 28% below the preradiation level. Also, there was a significant difference between the preradiation and postradiation histamine levels. These findings implicate histamine in the radiation-induced increase in IBF and HCT but not for the gradual decrease in postradiation blood pressure.

INCREASED URINARY EXCRETION OF HISTAMINE BY RATS FOLLOWING GAMMA RADIATION

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The effects of whole-body gamma radiation (900 rads) from a ^{60}Co source on hourly and daily excretion rates of urinary histamine (UH) were monitored in male (M) and female (F) Sprague-Dawley rats. Animals were housed in individual metabolic cages. Authentic UH (i.e. diamine oxidase degradable) histamine was determined for each sample. This was essential due to high non-specific fluorescence in rat urine. UH was measured fluorometrically after dialysis using a Technicon II antoanalyzer. Data were normalized to percent of control values obtained for each animal prior to radiation. UH in M and F urine were 0.58 $\pm$ 0.10 and 2.01 $\pm$ 0.18 ug/ml, respectively. M rats excreted 7.72 $\pm$ 1.22 ug UH/24 hrs while F rats excreted 27.2 $\pm$ 1.98 ug UH/24 hrs. There were no significant differences in urine volume (ml/24 hrs) between M and F rats. Increased excretion of UH in irradiated animals (F) (compared to sham-irradiated) was first observed 1 hr after radiation. UH levels continued to rise with a maximum UH excretion (195.5 $\pm$ 22.14% of preirradiated control levels) at 12 and 24 hrs. UH excretion returned to control levels 3 days after radiation. UH excretion in irradiated M rats followed a similar pattern. These results demonstrate an early radiation-induced increase in excretion of UH following whole-body gamma radiation which may reflect alterations in histamine release/metabolism in the irradiated animal.

MYOCARDIAL SCINTIGRAPHY WITH Tc-99m PYROPHOSPHATE AND GATED BLOOD POOL HEART FUNCTION STUDIES IN DOGS AFTER ACUTE IRRADIATION OF THE HEART

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Cardiac radiation damage has been described in various animal models as well as in human studies. Radiation induced heart injury in the experimental model was first suggested by Hartman in 1927 (1) followed by numerous reports describing wide tolerances to irradiation (2, 3, 4). More recent reports suggest acute cardiac morbidity dose is in the lower range (3000-7000 roentgens in the dog) than it was previously considered (5, 6).

In this work, we have studied the effect of a single dose of Co-60 irradiation (3000 and 6000 rad) on heart function and tissue damage by MUGA and Tc-99m PYP scintigraphic studies in male beagle dogs. Two groups of 3 beagle dogs were irradiated bilaterally with a single dose of cobalt-60 irradiation confined to the heart. The midline tissue dose rate was 57 rad/minute. Three dogs with isoproterenol induced myocardial infarct served as a model for the infarct-avid study.

Baseline Tc-99m pyrophosphate and cardiac function (MUGA) studies were obtained prior to the administration of isoproterenol or radiation. MUGA studies were performed on irradiated animals on day 3, 9, and 22 after irradiation and PYP studies on day 1, 6, and 8 after irradiation. The animals with isoproterenol induced heart damage received MUGA studies on day 2 and 7 and PYP studies on day 4 and 9 after drug administration. Control animals were sacrificed at parallel time intervals.

All animals were sacrificed intravenous administration of euthanizing agent (T-61). The heart was dissected out of the thoracic cavity; and tissue samples of the left and right ventricle, apex, septum, left and right atrium, left and right auricle, papillary muscle. aorta, pulmonary artery, and left and right lungs, were obtained and counted in a LKB Ultrogamma well counter. Statistical analysis was performed, and Tc-99m activity was expressed as a percent dose per gram of the tissue with a standard error of the mean. Electrocardiographic recordings were obtained on each animal during heart

function (MUGA) studies. All tissue samples were analyzed by histopathological examination.

The results demonstrate scintigraphic evidence of diffuse myocardial damage in isoproterenol injected dogs, as observed by the intensity of the uptake of 4/4+, on day 4 after isoproterenol administration. The study was negative on day 1 and day 8. Ejection fraction did not change significantly from the baseline values during 3 post-isoproterenol intervals.

ECG studies of isoproterenol treated dogs demonstrate acute myocardial damage on day 4 corresponding to the positive Tc-99m PYP scan. ECG data demonstrate chronic post-necrotic changes on day 8 after isoproterenol injection. Histopathological examination of the heart in the isoproterenol group demonstrates disseminated foci of myocardial necrosis with fibroplasia and inflammatory infiltrates. The tissue samples of different segments of the hearts of irradiated dogs showed no evidence of elevated Tc-99m PYP uptake in either the 3000 or 6000 rad group. Scintigraphic studies demonstrate no difference in the myocardial images between baseline and post-irradiation studies. Gated blood pool studies (MUGA) demonstrated no difference between the baseline (LVEF - 43.0 + 4.2) and the 3000 rad group on either day 3 (LFEV = 49.7 $\pm$ 0.3) or day 22 (LFEV = 52.5 + 13.5) post-irradiation. In the 6000 rad group there was no difference in LVEF between baseline (51.3 $\pm$ 1.7) and irradiated group (46.3 + 1.8), 9 days post irradiation.

Our results indicate that radiation induced changes in the heart of a dog irradiated with 3000 and 6000 rad include minimal to mild multifocal cardiac and pulmonary perivasculitis without necrotic changes by the histological examination. There was no evidence of radiation induced heart damage by either gated acquision heart studies, or by Tc-99m pyrophosphate scintigraphic studies in dogs in the early time periods after irradiation.

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EFFECT OF WHOLE-BODY IRRADIATION WITH PHOTONS AND FISSION NEUTRONS ON THE ORGAN DISTRIBUTION OF Ga-67 IN THE RAT

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The retention of gallium-67 (Ga-67) has been reported to be significantly decreased after acute whole-body irradiation. Swartzendruber and Hubner (Radiat. Res. 55 457-468, 1973) have observed this finding in mice and associated it with the radiation damage to the synthetic sites of a carrier molecule for Ga-67, in the cellular organelles such as lysosomes. Subsequent reports confirmed the finding of reduced whole-body retention of Ga-67 after irradiation in the rat, explaining it by an alteration in serum binding of Ga-67 after a single dose whole-body photon irradiation of 720 R. Bradley, et al, (J. Nuc. Med. 20 244-247, 1979) studied radiation effects on the distribution of Ga-67 in the rat and reported decreased tissue uptake of Ga-67 after irradiation. They postulated the association of radiation induced gallium decrease in the rat with increased serum iron levels and reduced unsaturated iron binding capacity resulting in decreased tissue uptake and increased urinary excretion of Ga-67.

In the present work we have studied the effect of a single dose whole-body irradiation with cobalt-60 (Co-60) and fission neutrons on the Ga-67 distribution in different organs of the rat at various intervals after irradiation. All experiments were performed on the female Sprague Dawley rats (250 g) maintained on a normal rat feed and water ad libitum.

Each of three groups of animals was irradiated with a specific dose of Co-60 photons (2, 4, and 6 Gy) at 0.4 Gy/min. Three other groups received whole-body fission neutron irradiation at 2, 4, and 6 Gy (Mark F TRIGA reactor). Irradiated animals were injected with 1.11 MBq Ga-67 citrate on the day of irradiation and 6, 14, 22, and 30 days after irradiation. Animals were sacrificed 48 hrs after Ga-67 administration; and the concentration of Ga-67 was determined in the blood, lung, heart, liver, spleen, kidney, adrenal, stomach, small and large intestine, ovaries, uterus, lymph nodes (right and left popiiteal, lumbar, and mediastinal) thymus, muscle, femur, and brain. The tissue samples were counted in a LKB Ultrogamma well counter. The results are expressed as percent dose Ga-67 per gram of tissue with a standard error

of the mean. Each time point of the experimental groups consisted of 5 animals. A group of five nonirradiated control animals was sacrificed by halothane anesthesia together with irradiated animals at each time interval and the distribution of Ga-67 was obtained in the same organs.

Our data demonstrate that whole-body photon radiation had little effect on the Ga-67 concentration in the examined organs on most of the experimental time intervals. Gallium concentration in the control animals was highest in the bone, followed by the liver, spleen, kidney, lymph nodes, and stomach. The lowest concentration of Ga-67 was observed in the brain, heart, and skeletal muscle. Most of the tissues were not affected in their Ga-67 uptake by either photon or neutron irradiation. The exceptions of significant decrease of Ga-67 were observed in the liver (2 and 4 Gy) on day 2 (6 Gy) on days 8, 16, and 24 after Co-60 irradiation ($p<0.05$), lung (6 Gy) 24 days, spleen (6 Gy) 32 days, stomach (4 Gy) 2 and 16 days, small and large intestine (6 Gy) 16 and 24 days post irradiation ($p<0.05$). An increase in Ga-67 uptake was observed in the mediastinal (6 Gy) 16 and 24 days, popliteal (6 Gy) 24 days and lumbar lymph nodes (4 and 6 Gy) 2, 6, and 24 days post-irradiation ($p<0.05$).

Neutron irradiated animals demonstrated no consistent differences in Ga-67 uptake when compared to the controls. The exceptions of lower Ga-67 uptake were noted in the lung (2 and 4 Gy) 16 and 24 days, stomach (4 Gy) 8 days, small and large intestines (2, 4, and 6 Gy) 2, 16, and 24 days, thymus (2, 4, and 6 Gy) 2 and 32 days, spleen (4 Gy) 8 days post-irradiation ($p<0.05$). Increased Ga-67 uptake was observed in popliteal lymph nodes (2, 4, and 6 Gy) 2, 16, and 32 days post-irradiation ($p<0.05$), lumbar (2 Gy) 16 days and mediastinal lymph nodes (4 Gy) 2 and 16 days post-irradiation ($p<0.05$).

These results demonstrate that Ga-67 distribution and quantitative tissue uptake are not consistently altered after photon or neutron irradiation at the doses of 2, 4, and 6 Gy in the 5 time intervals after irradiation. Our data indicate that pronounced decrease of Ga-67 in the tissue which has been described within 24 hours after irradiation (Fletcher, et al, 1975 Radiology 117 709-712, 1975) is not present at the radiation levels and the time intervals of our experiments.

Different authors have observed a sigrificant decrease of Ga-67 tissue uptake after acute whole-body irradiation. Discussion of the mechanisms of gallium decrease postulated the radiation effect on specific cellular organelles and synthetic sites of gallium carrier molecules (Schwartzendruber et al. 1973 Radiat. Res. 55 457-468, 1973). In our studies, neither Co-60 nor neutron irradiation produced a consistent alteration in gallium tissue uptake. This could be due to our longer experimental intervals after irradiation, compared to other studies where radiation effect on decrease of gallium uptake was observed. Our observations based on 48 hours uptake of Ga-67 at different time intervals after Co-60 or fission neutron irradiation suggest a possibility of the restitution of gallium uptake and transport mechanisms, known to be altered by acute whole-body irradiation.

Research was conducted according to the principles enuciated in the "Guide for the Care and Use of Laboratory Animals", prepared by the Institute of Laboratory Animal Resources, National Research Council.

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DIFFERENCES IN POPLITEAL NODAL UPTAKE OF Tc-99m ANTIMONY TRISULFIDE COLLOID AFTER COBALT-60 AND FISSION NEUTRON IRRADIATION

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Radiolabeled colloid (technetium-99m antimony trisulfide, Tc-99m ASC) has been used extensively for the clinical assessment of internal mammary lymph node involvement in patients with breast carcinomas (Int J Rad Oncol Biol Phys 2:755-761, 1977). In this study the effects of cobalt-60 (Co-60) and fission neutron irradiation (whole-body) on the ability of popliteal lymph nodes to retain TC-99m ASC were evaluated in male Sprague Dawley rats. Three groups of rats irradiated with different dosing schedules (6 Gy and 10 Gy Co-60 (0.4 Gy/min) and 6 Gy (0.4 Gy/min) fission neutrons) were studies at various time intervals ranging from day 0 to day 14 postirradiation.

In 1966 Dettman found that by using local irradiation (250 Kv x-ray) to the right popliteal lymph node, nodal distribution of Au-198 colloid in dogs was not significantly different from that of control values at doses 2000, 4000, and 10,000 R (Amer J Roent Rad Ther Nucl Med 96:711-718, 1966). Barrow noted that 800 R whole-body x-irradiation of rabbits did not interfere with Au-198 colloid uptake in reticuloendothelial cells (Amer J Physiol 164:822-831, 1951). In our study by injecting a different radiocolloid (0.55 MBq technetium-99m antimony sulfide colloid, Tc-99m ASC) via the footpad and using whole-body irradiation on rats, we observed a significant increase in nodal uptake of Tc-99m ASC after 6 Gy of Co-60 irradiation. Two to 14 days after the rats were irradiated with Co-60 (6 Gy, 0.4 Gy/min), uptake values of Tc-99m ASC by the left popliteal (LP) nodes (0.94 + 0.16 to 1.81 + 0.31 % dose/mg of node, mean + STD error) and the right popliteal (RP) nodes 0.82 + 0.09 to 1.31 + 0.35) increased significantly ($P<0.05$) as compared to the mean control values (LP = 0.38 + 0.04, RP = 0.47 + 0.13). For the 10 Gy Co-60 irradiated group of rats, the left nodal uptake values for Tc-99m ASC (0.79 + 0.12) were significantly higher compared to the mean control value (0.31 + 0.09) for only day 8 and not before ($P<0.05$). Nodal uptake values for the 6 Gy fission neutron group were not significantly different from the mean control values ($P<0.05$). Most of the mean popliteal nodal weights for the Co-60 irradiated rats were not significantly different from control values ($P<0.05$), but for the neutron irradiated group the mean nodal weight of the left popliteals on

day 2 (5.0 + 2.8 mg) and day 4 (3.0 + 1.7 mg) postirradiation were significantly less compared to the mean control value (14.5 + 7.71 mg, $P<0.05$).

The results indicate that Tc-99m ASC lymph node uptake was significantly increased in rats exposed to a lower dose of Co-60 (6 Gy) than in rats exposed to 10 Gy Co-60. In addition, fission neutron irradiation did not significantly alter the nodal uptake of Tc-99m ASC as compared to normal values. Sinha reported that after local irradiation of the popliteal nodal region of male Wistar rats with exposure doses of up to 20 Gy (250 Kv x-ray), phagocytic function of reticuloendothelial cells was still active although there was marked degeneration of lymphoid cells. Further examination of the lymph nodes revealed interstitial edema and widely dilated sinusoids (Cancer 26:1239-1244, 1970). The radiation in our study may be attributed to changes in macrophage phagocytic activity and/or structural alteration of the popliteal lymph nodes.

SUBLETHAL HEMORRHAGE IMPAIRS THE PERITONEAL INFLAMMATORY RESPONSE IN THE RAT

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The purpose of this study was to elucidate changes in the peritoneal inflammatory response to bacterial contamination after Hemorrhagic Shock (HS). Rats were subjected to an LD10 HS procedure consisting of 90 min of shock induced by withdrawing .03 ml of blood/gm body wt. Following reinfusion of the shed blood, 10^9 live E. coli were injected intraperitoneally. Animals were observed for mortality or killed at various times after injection of bacteria and peritoneal lavage performed. Sham Hemorrhaged (SH) rats served as controls. Mortality was 11% in the SH group compared with 67% in the HS group ($p<.05$). Immediately after innoculation of bacteria, the total number of leukocytes (expressed as cells x 10^6/ml) in lavage fluid (LF-WBC) was similar in both groups (SH:1.81 $\pm$ 0.14; HS:1.77 $\pm$ 0.15). LF-WBC was higher in the control group at 3 hrs (SH:3.11 $\pm$ 0.29; HS:2.08 $\pm$ 0.27; $p<0.05$) and 5 hrs (SH:3.53 $\pm$ 0.38; HS:1.33 $\pm$ 0.31; ($p<.01$) after the innoculation of bacteria. The number of polymorphonuclear leukocytes in lavage fluid was slightly higher in the controls at 3 hrs (SH:1.85 $\pm$ 34; HS:1.51 $\pm$ .56; $p>.05$) and significantly higher at 5 hrs (SH:2.82 $\pm$ .37; HS:1.02 $\pm$ .22; $p<.01$). There was no significant difference between the groups in the total number of circulating leukocytes at 0 hrs (SH:7.60 $\pm$ 1.36; HS:7.93 $\pm$ 1.52), 3 hrs (SH:7.35 $\pm$1.42; HS:6.12 $\pm$ 1.21), or 5 hrs (SH:4.31 $\pm$ .55; HS:4.29 $\pm$ .93). We conclude that HS impairs the migration of leukocytes into the peritoneal cavity of rats in response to bacterial contamination.

EFFECT OF SUBLETHAL IONIZING RADIATION ON MURINE PEYER'S PATCH LYMPHOCYTES

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After sublethal doses of ionizing radiation, murine Peyer's Patches lymphocytes regenerated significantly more slowly than lymphocytes from spleen, thymus, and peripheral lymph nodes. Long Evans rats were exposed to 150 rads (40 rads/min) of whole-body irradiation from a Cobalt-60, gamma-emitting source. On days 1-20 postirradiation, single-cell suspensions of lymphocytes from thymus, spleen, peripheral lymph nodes, and Peyer's Patches were stained with monoclonal antibody reagents to detect Ia-positive cells, nonhelper T-cells, and helper T-cells. Cells were then counterstained with Texas Red conjugated anti-mouse IgG and were also stained with fluorescein diacetate to determine the viability of lymphocytes. The percentages of viable lymphocyte populations were analyzed using a dual-laser, fluorescence-activated cell sorter (Becton-Dickinson FACS-II). We observed that viable lymphocyte populations in thymus, spleen, and peripheral lymph nodes from irradiated animals returned to normal (nonirradiated control animals) levels 3-7 days postirradiation, while viable lymphocyte populations in Peyer's Patches from irradiated animals remained suppressed up to 20 days PI. These results suggest that either the lymphocytes or, more likely, the microenvironment of Peyer's Patches is more greatly damaged by ionizing radiation than that observed in other lymphoid tissue.

THE PATHOGENESIS OF EXPERIMENTAL CNS RADIATION INJURY AND ITS RELATIONSHIP TO "METHOTREXATE ENCEPHALOPATHY"

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Eighteen male rhesus monkeys received 1800 rads to the whole brain within 8.5 minutes. Nine of the monkeys received prophylactic intramuscular dexamethasone beginning 1.5 days prior to radiation and continuing on a daily basis for a total of 12 days. Twelve animals were perfused with Karnovski's solution; 5 of these animals also received intravenous horseradish peroxidase while most of the animals received intravenous Evan's blue prior to sacrifice. A triad of white matter lesions emerged including: (1) widespread, patchy edema and secondary demyelination, (2) focal necrosis, (3) focal hemorrhage. The Evan's blue dye and horseradish peroxidase marker documented abnormalities of vascular permeability related to primary injury of the endothelial cells. We also observed evidence of extensive axonal damage, reflected by the presence of calcified axonal bodies were remarkably similar to those described in human "methotrexate encephalopathy". Conclusions: (1) dexamethasone does not protect the CNS from delayed radiation injury, (2) the endothelial cell represents the primary target of radiation injury, (3) the morphologic changes are similar, if not identical to those described interalia as "methotrexate encephalopathy".

PHENOTYPIC CHANGES IN LYMPHOCYTE SUBSETS IN THE FIRST WEEK AFTER SEVERE MULTIPLE TRAUMA

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Studies in our laboratory have confirmed that peripheral blood lymphocytes from multiple trauma victims respond poorly to the mitogen phytohemagglutinin in the first days following trauma. Patients with head injuries have been excluded from this analysis as they (3/3) appear to have normal responses. The average response of the remaining 10 patients, who had all been hospitalized in the surgical intensive care unit at Boston City Hospital, was 26 $\pm$ 6% compared to normal control responses measured in the same assay.

Patient cells were also evaluated for their distribution within lymphocyte subsets using monoclonal antibodies. All patients were studied for the distribution of T4 (helper/inducer) cells, T8 (suppressor/cytotoxic) cells, and T11 (a pan-T cell marker which recognizes the sheep red cell receptor and is also present on a subpopulation of NK cells). It was found that the average value for the patient T4 population was 31 $\pm$ 4% positive, compared to 52 $\pm$ 4% for the controls. This represents a highly significant decrease. In contrast, the percentage of T8 positive cells did not differ significantly from normals. The average patient value for T8 was 20 $\pm$ 4% compared to 22 $\pm$ 2% for normals. As a result of the decrease in T4 cells an imbalance in the T4/T8 ratio was observed. The patient T4/T8 ratio was 1.7 $\pm$ .3 compared to a control value of 2.7 $\pm$ .3. The pateint T4/T8 ratio was calculated excluding one patient whose T4 was 16% and T8 was 1%. The value for T11 was also low (53 $\pm$ 6% for patients compared with 78 $\pm$ 8% for controls). These results have certain parallels to those reported for the AIDS patients.

To determine the phenotype of the cells which were in one sense replacing the patients T4 cells, the panel of antisera used to study cells was expanded to include markers for B cells (B-1), NK cells (leu-7), immature lymphocytes and activated T cells (T10), and I-2 (a DR determinant found on B cells and activated T cells). Preliminary results indicate none of the subpopulations defined by these markers are elevated in trauma victims. The data therefore

indicate that these patients have a relative increase in the population of null cells. The lack of increased T10 or I-2 expression further suggests that the increase of null cells is not a result of an influx of immature cells, nor a predictable result of cell activation. While these results are intriguing, it should be mentioned that these changes in phenotypic expression do not seem to correlate with immunocompetence as measured by the ability to respond to PHA, nor with the development of sepsis. These findings may offer new insights into underlying mechanisms whereby the immune system is compromised in trauma patients.

THE EFFECT OF GAMMA RADIATION ON MAST CELLS IN VITRO

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As the initial step in examining the effects of radiation on the secretory response, we studied the effects of gamma radiation on histamine release (HR) in the rat peritoneal mast cell (RPMC). Radiation damage was monitored by measurement of spontaneous HR, trypan blue exclusion, membrane lipid fluidity, and the response to the HR agent, cpd. 48/80. Purified RPMC suspensions were exposed to a range of gamma (^{60}Co) radiation up to 1000 Gy at 4°C. Spontaneous HR during irradiation was only slightly higher than that of controls. However, the HR response stimulated the cpd. 48/80 was inhibited in irradiated cells. Above 50 Gy, cells exposed to 1.0 ug/ml cpd. 48/80 for 5 min at 37°C showed a dose related inhibition of HR which diminished to 5.0 $\pm$ 0.5% at 1000 Gy (controls: 49.0 $\pm$ 1.1%). The decreased response in irradiated cells does not appear to be related to a loss of cell viability. Membrane fluidity was measured in irradiated RPMC using 16-doxyl stearic acid, a lipid spin-label probe. No significant changes in EPR parameters were observed over the radiation dose range, compared to controls. The data demonstrate that gamma radiation (to 1000 Gy) does not induce spontaneous HR in vitro. Radiation above 50 Gy inhibits cpd. 48/80 HR. This phenomenon does not appear to be correlated with changes in cell viability or membrane fluidity.

PRELIMINARY STUDIES ON THE EFFECTS OF GLUCAN-P AND GLUCAN-F ON CANINE HEMOPOIESIS

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Glucan is a B-1,3 polyglucose isolated from the inner cell wall of the yeast Saccharomyces cervisiae. It is a potent immunologic and hemopoietic modulator. We have previously shown that both particulate (glucan-P) and soluble (glucan-F) glucans enhance hemopoiesis in normal and in hemopoietically depressed mice. Recently, it was decided to assay the hemopoietic effects of glucan in a more clinically relevant model--the canine. Normal female beagles were intraperitoneally administered either 7.5 or 30.0 mg of glucan-P per kg of body weight. One, 2, 3, 4, 7, 10, 14, 17, 21, 28, 35, and 42 days later, peripheral white blood cellularity (RBC), and platelet counts were performed. In addition, the numbers of granulocyte-macrophage (GM-CFC) and erythroid (CFU-e) progenitor cells per 10^5 nucleated bone marrow cells were determined by in vitro culture assays. One day post-treatment, peripheral blood WBC counts peaked with the greater response being elicited by the higher glucan dose (250% of baseline values). This initial response subsided by day 3; however, a smaller secondary increase in WBC numbers was observed between days 4 and 21 post-treatment. In the bone marrow, granulocyte and macrophage progenitor cell (GM-CFC) numbers also initially increased (peak on day 2 post-treatment). The GM-CFC increase, however, was followed by a decrease in GM-CFC numbers that fell below baseline values. Peripheral blood RBC counts did not deviate from baseline values until day 7 post-treatment. At this time, RBC counts fell only slightly below baseline values and remained decreased until 28 days post-treatment (maximum decrease of 20%). Contrary to peripheral blood RBC counts, bone marrow erythroid progenitor cells (CFU-e) initially decreased in numbers from days 1-10 post-treatment. This decrease was followed on days 21 and 28 post-treatment by a significant overshoot of baseline values. Similar to the response observed with bone marrow CFU-e, peripheral blood platelets also decreased in number (10%-40% decrease on days 1-4 post-treatment), followed by an overshoot of baseline values. All cellular responses elicited by glucan-P were temporary and had completely returned to normal baseline levels by 5 weeks post-treatment. In addition to these cellular hemopoietic responses, the appearance of C-Reactive Protein (C-RP, an acute-phase reactant) was measured in the serum of

glucan-P-treated dogs. C-RP peaked on day 1, fell sharply by day 4, and then gradually continued to decrease. Baseline values were not obtained until 28 days post-treatment. These results will be compared to the hemopoietic responses we are currently eliciting in canines with glucan-F treatment.

POSTRADIATION PLASMA GLUCOSE VARIATIONS

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Radiation-induced hypotension in the canine is accompanied by increased intestinal submucosal blood flow and increased hematocrit. This study was performed in order to correlate this radiation-induced hypotension with plasma glucose levels following radiation. The glucose levels were monitored in the systemic arterial circulation at the level of the abdominal aorta and in the hepatic portal vein before and after radiation. Plasma glucose was determined on a Beckman Glucose Analyzer which employs the enzymatic reaction of β-D-glucose and oxygen. Concurrent with postradiation hypotension, we measured a distinct decrease in plasma glucose in both the systemic arterial circulation and in the hepatic portal vein. This decrease showed a significant decrease at approximately 20 minutes postradiation and continued for the 60-minute interval of measurement. When control mean blood pressure and plasma glucose data were tested for correlation using glucose levels from both the aortic plasma and hepatic portal vein plasma, there was no correlation between blood pressure and glucose levels ($r = -0.393$ and 0.133, respectively). However, when the same test was performed using data from the radiated animals, a distinct correlation was noted between systemic mean blood pressure and aortic plasma glucose levels ($r = 0.975$) and also between systemic mean blood pressure and portal vein plasma glucose levels ($r = 0.983$). These correlation coefficients lead us to conclude that these two physiological parameters may be interrelated. Likewise, the significant difference ($p = 0.05$) between control and radiated animals leads us to conclude that radiation is somehow responsible for the positive correlation.

GENETIC DIFFERENCES IN THE MAGNITUDE OF ACUTE PHASE SERUM AMYLOID P AND A (SAP, SAA) RESPONSES OF MICE TO ENDOTOXIN

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Macrophages appear to play a role in the acute phase elevation of murine serum amyloid P (SAP) concentration as well as in that of serum amyloid A (SAA), when responder and nonresponder C3H mice are injected with protein depleted lipopolysaccharide (LPS) (Ann NY Acad Sci 389, 137, 1982). In this study we compared the pattern of acute phase increases in SAP and SAA in 11 strains to determine whether there is a direct correlation between acute phase increases in SAP and SAA. Groups of 6 female mice, 9 to 12 weeks old, were injected i.p. with 1 ug of E. coli LPS K235 (ph) and serum was obtained 24 hours later. Normal controls were untreated. SAA and SAP concentrations were measured by radioimmunoassay and analyzed by the Student t test. There was no direct correlation between the geometric means of SAP and SAA responses ($p < .001$). Three categories of SAA responders were defined by comparing the geometric means of normal and acute phase SAA concentrations.

Strain		SAA (ug/ml) Normal	SAA (ug/ml) Acute Phase	SAP (ug/ml) Normal	SAP (ug/ml) Acute Phase
Balb/cJ		2	161	32	72
A/J		3	95	30	55
CBA/J	High	1	88	20	57
SJL/J		1	76	13	62
DBA/J		1	47	41	89
AKR/J		1	26	22	94
C3H/HeCR	Medium	1	24	36	55
C57/Bl/GJ		5	24	17	38
CD-1/CR		1	14	22	79
SWR/J		1	12	15	45
C3H/HeJ	Low	1	4	21	30

When the differences among the strains were analyzed by Scheffe's test, the acute phase SAA concentration of 5 strains designated high responders was significantly different from the low responder C3H/HeJ mice ($p<.001$). Those strains for which there are no significant differences between either high or low are designated medium responders. However, the highest SAA responder Balb/cJ was also significantly different from the two medium responders CD-1/CR and SWR/J, ($p<.001$). The acute phase concentration of SAP in C3H/HeJ

mice was the lowest of the 11 strains, and was significantly lower than 3 high SAA responsders DBA/J, Balb/cJ, and SJL/J, and 2 medium SAA responders AKR/J and CD-1/CR($p<.001$).

We conclude that the magnitude of acute phase SAA and SAP elevations is controlled both by the host and by the dose of inflammatory stimulus, and that the mechanisms of SAA and SAP elevations are in part dissociable. This is further supported by the observation that BCG infected C3H/HeCR mice exhibited a much greater SAA response to 1 ug LPS than could be achieved with as much as 100 ug of LPS in uninfected mice.

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